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AD

Report No. 2.

CHEMICAL STUDIES
IN CONNECTION WITH
POTENTIAL SYSTEMIC INSECT-REPELLENTS AND PROPHYLACTIC AGENTS DEPOSITED IN THE SKIN

Progress Report

by

R. P. Quintana, Ph.D., Associate Professor of Medicinal Chemistry and Principal Investigator
A. Lassio, Ph.D., Professor and Chairman, Department of Medicinal Chemistry

August 196[illegible]

U. S. Army Medical Research and Development Command
Office of the Surgeon General
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Department of Medicinal Chemistry
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Research Contract No. DA-49-193-MD-2636
Task 01, Mil. Med. Res. Program S.E. Asia, Malaria Investigations
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FOREWORD

The project CHEMICAL STUDIES IN CONNECTION WITH POTENTIAL SYSTEMIC INSECT-REPELLENTS AND PROPHYLACTIC AGENTS DEPOSITED IN THE SKIN was authorized under Contract No. DA-49-193-MD-2636 (Project No. 3A025601A811-01, Task 01, Mil. Med. Res. Program S.E. Asia, Malaria Investigations) and was activated 1 September 1964.

This Report covers the period 1 September 1965 through 31 August 1966, and constitutes the second annual progress report.

The information in This Report has not been cleared for release to the general public. The findings contained therein are not to be construed as an official Department of the Army position, unless so designated by other authorized documents. According to current regulations, reproduction of copyrighted material by the United States Government is not permitted.

We would like to acknowledge the capable assistance of Miss Eleanor D. Yeaglin, M.S., and Mrs. Pamela P. Boggs, B.S., in the synthetic phase of our program, and that of Mrs. Sandra L. Ousley, B.A., in the physicochemical aspects of our work, all of our staff.

Relative to the evaluation of the insect-repellency of our

synthetic entities. we wish to acknowledge the continued interest and assistance of Doctor Carroll N. Smith (Investigations Leader, Entomology Research Division, U.S. Department of Agriculture). In addition, we would like to express our appreciation to Mr. Philip Kashin (Associate Biochemist, Life Sciences Research, IIT Research Institute) for his contribution to our studies in evaluating the insect-repellent properties of our compounds.

Regarding the dermatological aspects of our program, we gratefully acknowledge the counsel of Doctor Robert G. Crounse (Chairman, Division of Dermatology, Johns Hopkins University School of Medicine) and of Doctor Eugene J. Van Scott (Director of Intramural Research, National Cancer Institute). We are particularly pleased that Doctor Crounse will co-operate with us even more closely beginning September first.

In connection with our studies involving monomolecular films, we wish to acknowledge the enthusiastic help of Doctor Herman E. Ries, Jr. (Senior Research Associate, Research and Development Department, American Oil Company).

We wish to offer again our sincere gratitude to Brig. General C. F. Vorder Bruegge and his staff for their unstinting support, interest and assistance throughout the past year.

SUMMARY

The subject investigation constitutes a continuation of our previous studies[1] directed toward the development of reliable prophylactic agents against pathogenic or physical impairments inflicted through the skin. While our experimental design encompasses studies which are anticipated to yield information for the development of moieties possessing keratinization-enhancing, sunlight-induced-erythemogenesis-preventing, antidermatophytic and antibacterial properties, our primary targets are dependable and safe agents with long-lasting insect-repellent efficacy.

In order to accomplish the objectives set forth above, we have undertaken studies on the structural requirements and physicochemical characteristics associated with a compound's capacity (a) for anchoring to dermal tissue constituents following its topical administration, and/or (b) for enhancing its localization in dermal tissue following its parenteral, and even peroral, administration. It is our endeavor to integrate either of these, with molecular features known to possess insect-repellency, into new hybrid molecules. These new synthetic moieties would be expected to be long-lasting insect-repellent agents whether they be applied topically or administered systemically.

The reader is referred to the preceding report[1] and

published communication[2] (see Appendix) for supplemental information.

In conformance with these considerations, within the period covered by This Report -

- (a) The information developed in our laboratories during the preceding year was supplemented with exhaustive literature surveys, and the premises of our rationale delineated in our Report No. 1.[1] were reinforced and expanded.(Part One.)

- (b) Six additional new target compounds were synthesized, characterized and their postulated structures confirmed (Part Two). The novel hybrid molecules, reported for the first time, are:

  Tetrahydropyran-2-yl Ester of 4-(10-Carboxydecyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (compound A028),

  4-(10-Carboxydecyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (compound A029),

  Tetrahydropyran-2-yl Ester of 4-(9-Carboxynonyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (compound A032),

4-(9-Carboxynonyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (compound A033),

Tetrahydropyran-2-yl Ester of 4-(7-Carboxyheptyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (compound A035),

4-(7-Carboxyheptyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (compound A036).

Additional quantities of the first six new compounds, reported in the preceding communication,[1] were prepared for further insect-repellent and antidermatophytic evaluations.(Part Four.)

- (c) Instrumentation has been further refined and the previously described techniques[1] have been advanced permitting use of a uniform monomolecular-film system for the evaluation of all compounds studied in our laboratories. The interactions of our synthetic entities with stearic acid, cholesterol, lecithin and other skin constituents have been, or are expected to be, studied. We consider ourselves fortunate that our very first attempts in designing the proposed new hybrid molecules yielded compounds some of which registered measurable interaction with substances known to be components of, and to have prominent functions in, dermal tissue.(Part

Three.)

- (d) The insect-repellent properties of our compounds were studied by Doctor Carroll N. Smith (Investigations Leader, Entomology Research Division, U.S.D.A.) and Mr. Philip Kashin (Associate Biochemist, Life Sciences Research, IIT Research Institute). Here, too, our initial efforts resulted in novel compounds, some of which, among those already evaluated, registered measurable insect-repellent characteristics. While we are most interested in developing agents surpassing in effectiveness those currently in use, immediately, we are concerned with the relative potency of our synthetic entities with respect to each other; the importance of this approach in finding significant leads to the solution of the more practical aspects of the problem cannot be overestimated. We are therefore not particularly concerned, at this time, with the fact that none among our <u>first</u> six compounds[1] yielded favorable results in U.S.D.A.'s <u>standard</u> mosquito-repellent screening test for practical application.(Part Five.)

- (e) The efficacy of our compounds in inhibiting dermatophytic organisms was evaluated by Doctor Robert G. Crounse (Chairman, Division of Dermatology, Johns Hopkins University School of Medicine). It is salient

that, in this instance also, rather significant responses were observed. Among our compounds, those already evaluated required a higher concentration than the standard antidermatophytic agent. In addition to considering that we are dealing with the products of the initial phase of our exploratory efforts, and are primarily seeking leads, we feel that, if our compounds should possess enhanced localization characteristics in dermal tissue, the latter could render them therapeutically more effective. Doctor Crounse is also in the process of determining the range of the spectrum of antidermatophytic activity for each of our compounds. (Part Six.)

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PART ONE

RATIONALE

As indicated in the preceding report,[1] it is our endeavor to develop reliable prophylactic agents against pathogenic or physical impairments inflicted through the skin, which would have longer lasting effects, and thereby considerably reduce the frequency of their administration, as well as permit more effective prophylactic control within the various organizational structures of our armed forces. Our experimental approach encompasses studies designed to produce data essential for the synthesis of organic compounds possessing keratinization-enhancing, sunlight-induced-erythemogenesis-preventing, antidermatophytic and antibacterial properties. At the present time, however, our attention is focused primarily on dependable and safe agents with long-lasting insect-repellent efficacy.

In regard to the latter, we have undertaken studies on the structural requirements and physicochemical characteristics associated with a compound's capacity (a) for anchoring to dermal tissue constituents following its topical administration and/or (b) for enhancing its localization in dermal tissue following its parenteral, and even peroral administration. It is our endeavor to integrate either of these, with molecular features known to possess insect-repellency, into

new hybrid molecules. The products of these efforts would be novel synthetic entities, expected to have long-lasting insect-repellent efficacy whether they be applied topically or administered systemically.

It is fairly well known that among the currently available insect-repellents, even the best lose effectiveness relatively rapidly after topical administration due to absorption, evaporation and removal by solids and liquids one ordinarily comes into contact with.[3] It is comparatively unknown, however, that none of the effective repellents are resistant to removal by water, even under relatively mild exposure.[4]

It is obvious that one of the major objectives -whether we talk about topically applied or systemically administered agents- is to determine structural characteristics which render a molecule capable of anchoring to, or localizing in, dermal tissue.

Figure 1 illustrates the complexities of systemic administration, which may include losses (a) due to possible metabolic changes in the compound and/or (b) due to unanticipated possible accumulation of the compound in non-target body tissues or fluids.

In view of the immediacy confronting those associated with the overall program and since, as stated earlier, elucidation of the nature and mechanics of interaction with dermal tissue is of prominent importance to the development of an effective topical, as well as systemic, insect-repellent agent, our immediate emphasis is placed upon the preparation of compounds which, although topically applied, would exert long-lasting insect-repellent action due to their concurrent interactivity with skin.

In implementing this objective, one could approach the problem in two ways: (1) the preparation of the hybrid molecule delineated in several of the preceding paragraphs (see Figure 3); and (2) the synthesis of a compound arrived at by the method outlined in Figure 4. In the latter instance, the "precursor molecule" may or may not possess repellent properties in its existing form. However, upon intimate exposure to the epidermal surface, under the influence of pH, or enzymes, or bacterial flora, or the combination of all three and possibly other factors, the "precursor molecule" would release, at a very gradual pace, the active insect-repellent moiety.* If we consider that the specific rate of

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*By way of comparison, it has been stated that triacetin (glyceryl triacetate) exerts its antifungal effect, when applied topically, via release of acetic acid.[5]

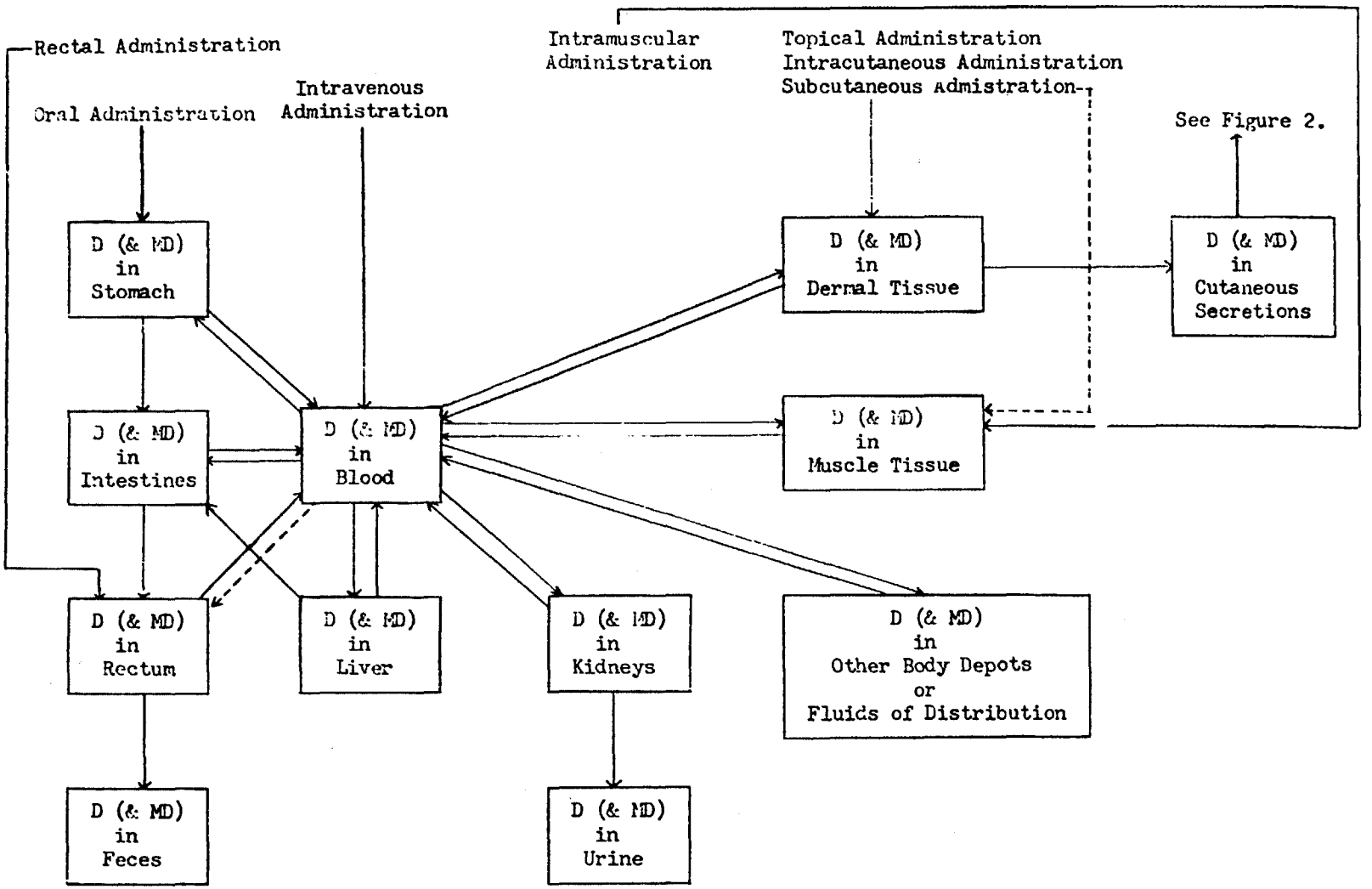


Fig. 1.— Representation of some pathways of drug administration, absorption, distribution, metabolism and excretion. D = drug; MD = metabolized drug.

1. DIRECT DIFFUSION INTO EPIDERMIS AND/OR INCORPORATION INTO EPIDERMAL PROTEINS.	2. VIA SWEAT GLANDS.	3. VIA SEBACEOUS GLANDS.	4. INCORPORATION INTO HAIR (AND NAILS).
From blood-vessel to connective tissue to basal epidermal cells to surface as cells divide and migrate to surface.	From blood-vessel to connective tissue to sweat gland to surface with secretion.	From blood-vessel to connective tissue to sebaceous gland to hair follicle to surface of skin and hair.	From blood-vessel to connective tissue to hair follicle to hair.
Rate independent of temperature.	Rate highly dependent on temperature.	Rate not temperature dependent, nearly constant, relatively rapid.	Rate not temperature dependent. Slow.
Time to reach surface variable. May be as much as 60 - 90 days.	Mainly water-soluble materials.	Mainly lipid-soluble materials.	Material located only in hairs (and nails).

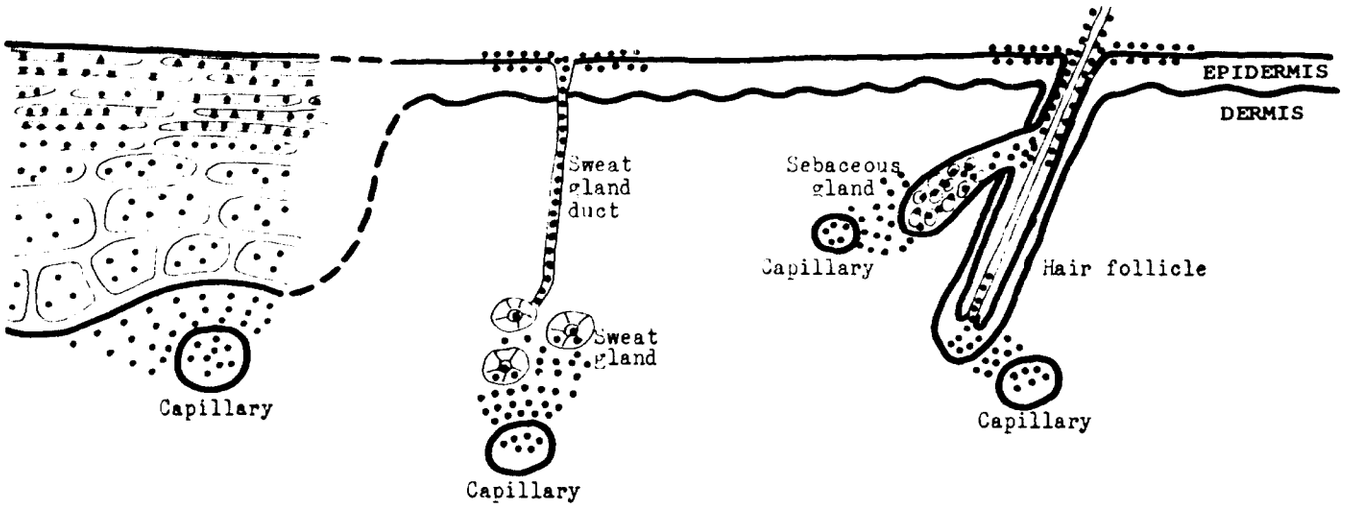


Fig. 2.— Routes of material from blood to surface of skin. (L. R. Fitzgerald, Ph.D.)

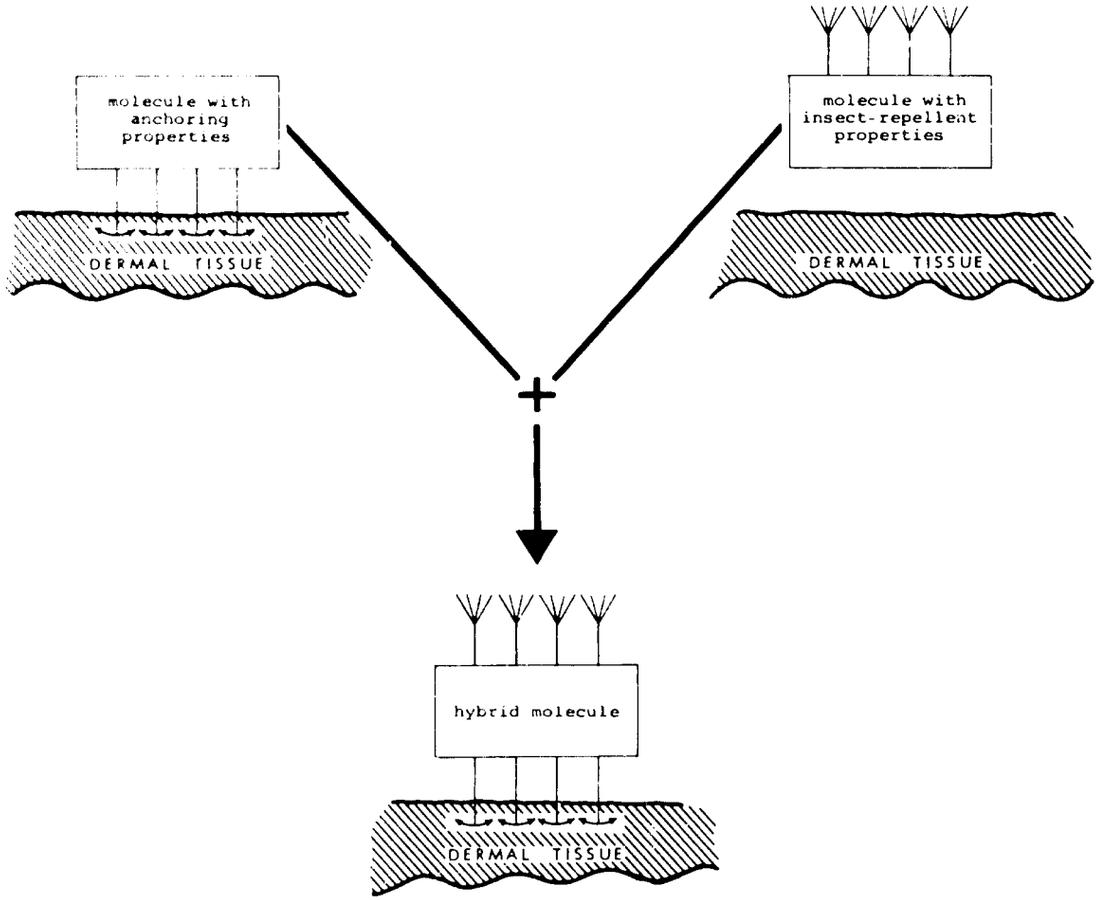
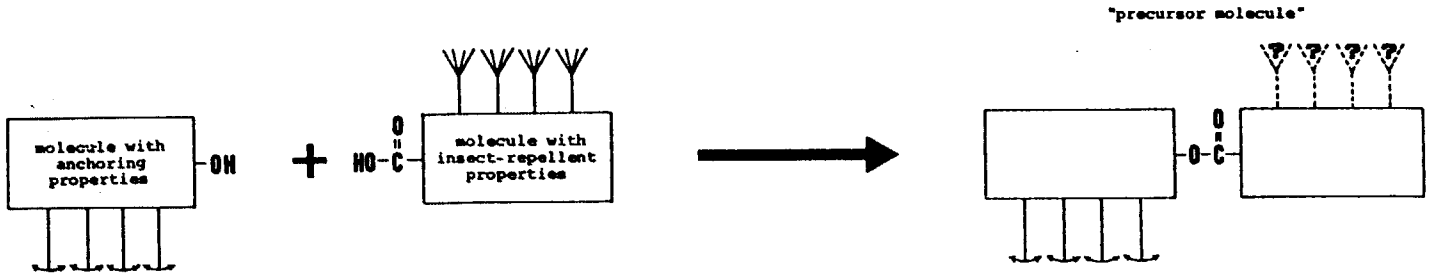
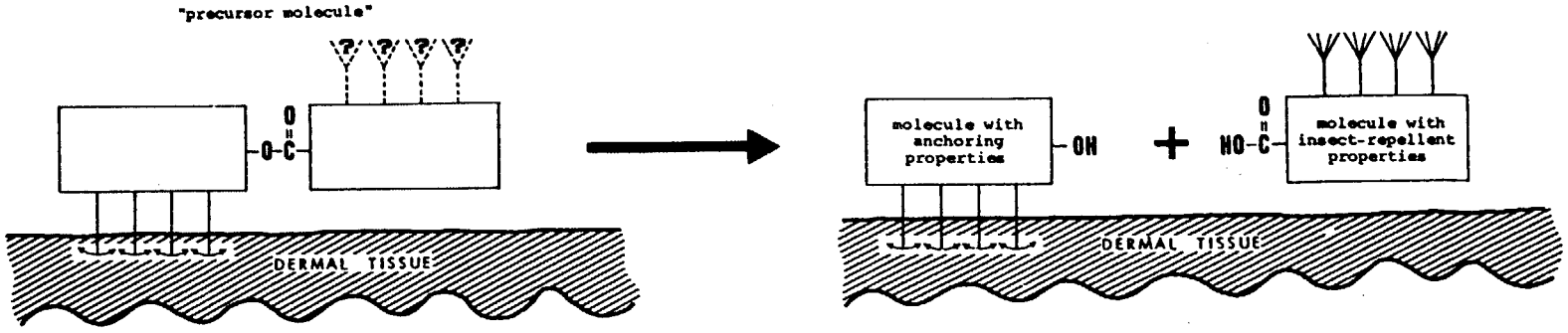

molecule with anchoring properties
molecule with insect-repellent properties
DERMAL TISSUE
DERMAL TISSUE
hybrid molecule
DERMAL TISSUE

Fig. 3



SYNTHETIC PREPARATION



UPON EXPOSURE TO EPIDERMAL SURFACE GRADUAL RELEASE OF ORIGINAL INSECT-REPELLENT MOLECULE

Fig. 4

release of the active repellent could be regulated by incorporating into the "precursor molecule" appropriate steric and/or electron-distribution factors,[6] the potentialities of this approach assume indeed significant importance. While the specific example in Figure 4 depicts an ester bond linking the anchoring component to the insect-repellent component of the molecule, other types of chemical connection could be also employed.

The monomolecular-layer technique, adapted to the purposes of our evaluation, affords a relatively rapid *in vitro* screening procedure for the qualitative detection, determination of the nature, and quantitative evaluation of the extent of interaction between (a) our series of compounds and (b) representative dermal elements[7a,8-13] or pertinent related materials. Whenever necessary, these studies are expected to be complemented with *in vivo* dermal localization experiments by Doctor Robert G. Crounse (Chairman, Division of Dermatology, Johns Hopkins University School of Medicine).

In the method we are now using a film, containing a mixture of the synthetic compound evaluated and a purified skin component, is deposited on an aqueous substrate. The film is compressed to its collapse point, and the pressure-area curves obtained from the experimental data are compared with

those obtained for the corresponding purified skin component alone.

Some of the various types of pressure-area isotherms which may result from studies of these types are shown in Figure 5. Using these hypothetical curves, we may illustrate the types of information that may be derived from studies of this nature. In each case, comparison will be made with the pressure-area curve for the pure skin component (i.e., curve A).

If the test compound deposited, in mixture with the purified skin component, has no affinity for this substance and/or is sufficiently water soluble, it will dissolve in the substrate, and the resulting pressure-area curve would be identical with that of the pure skin constituent itself (curve A).

Curve B illustrates a case where the test compound remains in the monolayer, as evidenced by the increase in the area-per-molecule parameter. In this instance, the chemical attraction between the two components in the film is such that the film collapses as a uni., at a pressure greater than that of the pure skin constituent.

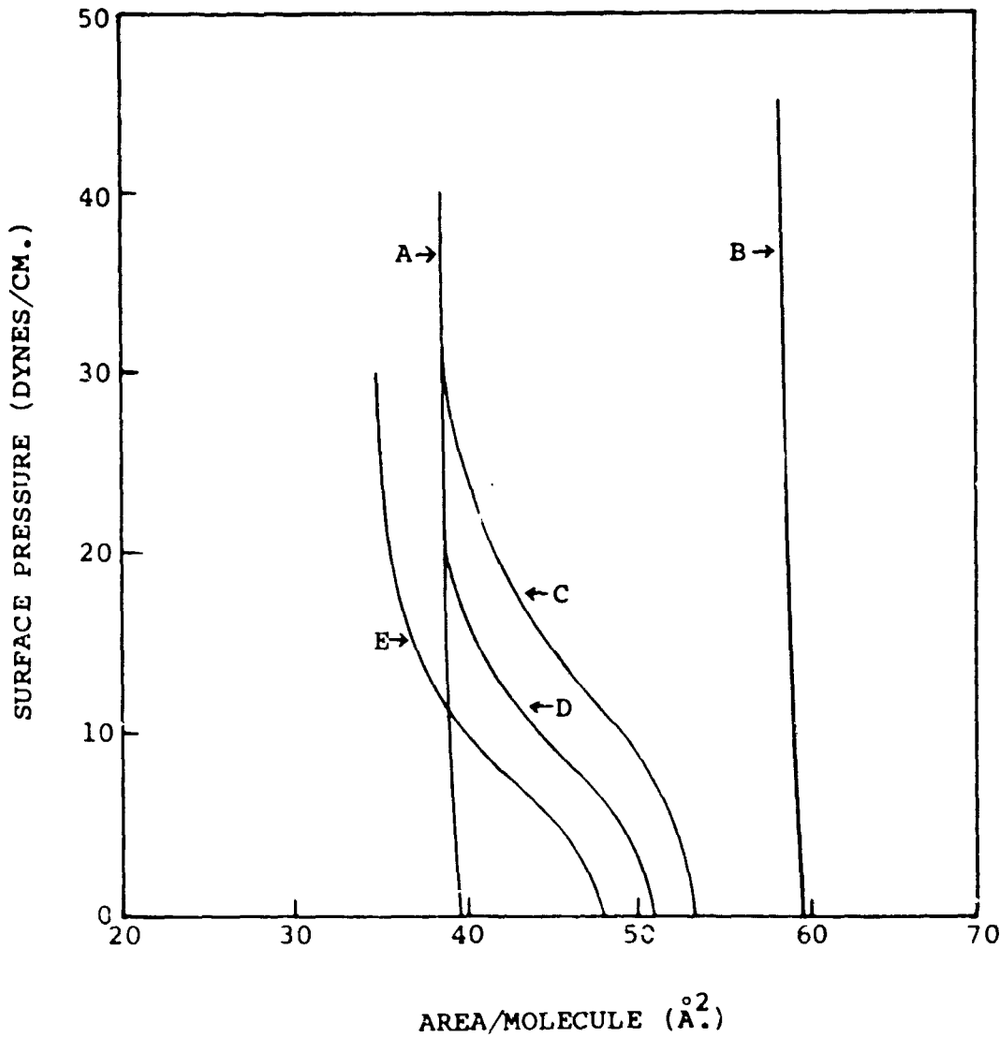


Fig. 5.— Typical pressure-area curves obtained in monomolecular-layer studies on mixed films.

Curves C and D are similar in that the test compound, in each case, is initially retained in the film (i.e., at lower pressures), but is squeezed from the area-determining portion of the film at the point where the curves intersect the curve of the pure skin component (A). The pressure at which the test compounds are ejected from the film may be taken as a measure of the affinity the evaluated synthetic compound and the purified skin component have for each other.[14] In this illustration, the difference in ejection pressure is 10 dynes/cm., and one might conclude that the test compound involved in curve C possessed significantly greater affinity for the skin constituent than did the test compound represented by curve D.

Curve E represents another case where the test compound is initially retained in the film and is later forced out as pressure is increased. In this instance, the fact that the vertical portion of the curve (at higher pressures) lies to the left of curve A, signifies that some of the skin-component material has been removed from the area-determining position, possibly due to "interfacial dissolution."[15]

While, in experiments of this type, absolute values are important to us, we are primarily interested in the relative degrees of effectiveness produced by our series of closely

related derivatives. These relative values are the ones which provide the most effective leads to the practical solution of our problem.

In considering the chemical aspects associated with the implementation of our objective, griseofulvin was selected, as the first of several entities, to contribute the dermophilic characteristics associated with its tendency of migrating to, and localizing in, dermal tissue.[16] In synthesizing our target compounds we used, first, griseofulvin's molecular structure to plan modified moieties and derivatives in such a manner that potential differences in the behavior between the member compounds of the series should assist in the identification of structural features of molecules associated with affinities for absorption by basal and/or adsorption to horny integumental cells. For similar reasons, synthetic entities were designed incorporating the structural features of undecanoic, decanoic and octanoic acids. The association of the latter two with natural dermal functions is well known.[7b,17] The antidermatophytic nature of all parent compounds cited is well established.[18-20] The acids, in addition, have been found to possess superior insect-repellent properties.[21]

It should be emphasized that the compounds named above are only some of those we expect to use in our investigation,

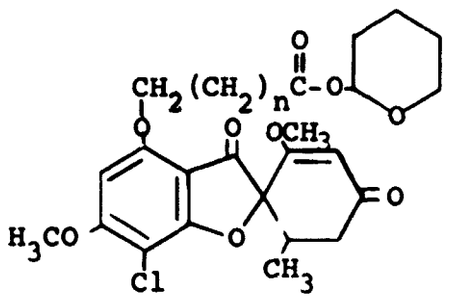
and are expected to serve as an indication of our approach rather than be considered an inclusive listing.

For example, among the other compounds on the drawing board are dihydroxyacetone[22-28] and hexachlorophene[29-30] which seem to be particularly promising in contributing means for anchoring molecules to the epidermal surface.

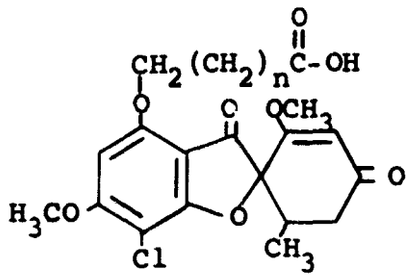
PART TWO

SYNTHESIS, CHARACTERIZATION AND PROOF OF THE MOLECULAR STRUCTURE OF NEW TARGET COMPOUNDS.

In accordance with the rationale described in Part One of This Report, we have synthesized the series of new, potential systemic insect-repellents shown below.*



A028, n = 9
A032, n = 8
A035, n = 6



A029, n = 9
A033, n = 8
A036, n = 6

The method employed to prepare these derivatives is illustrated in Figure 6 in which the synthesis of compound A029 is specifically shown. The method entails (1) protection of the carboxyl group of 11-bromoundecanoic acid (Y003) by conversion

*The reader is also referred to the Formula Review in the Appendix for the structures of these and other pertinent compounds.

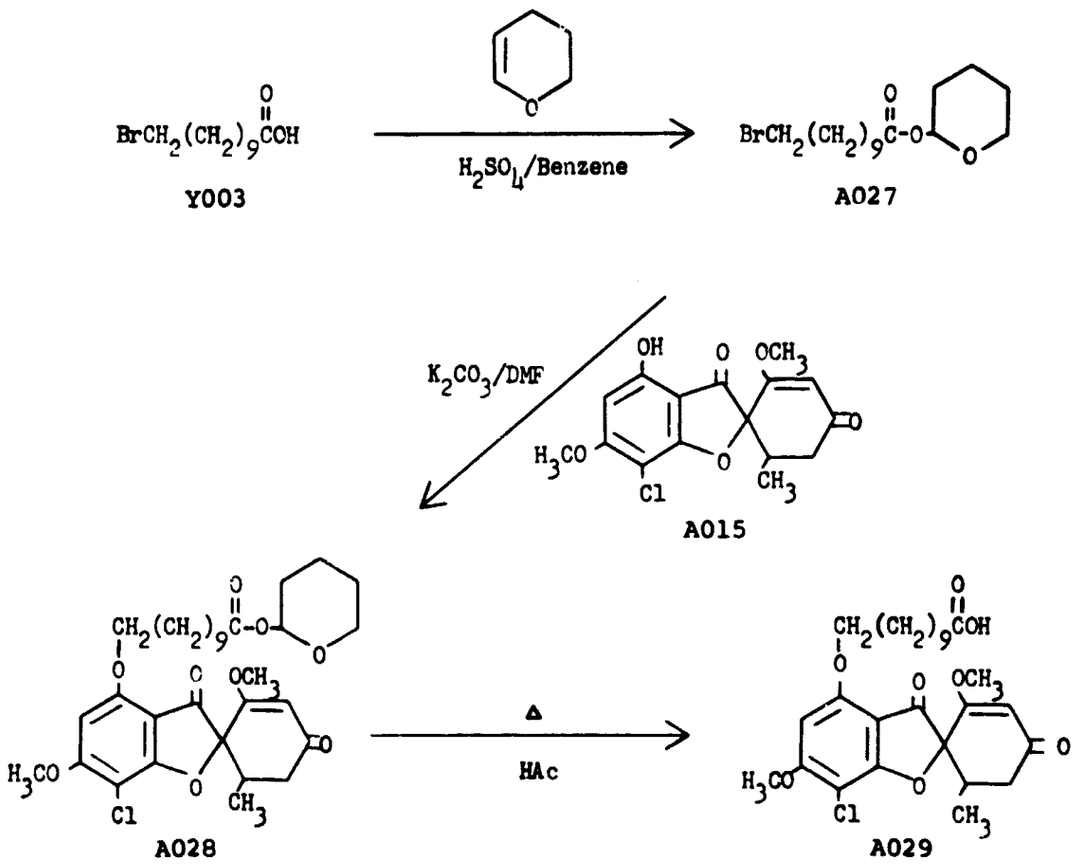


Fig. 6.— Method employed for synthesizing new target compounds: preparation of 4-(10-carboxydecyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (A029).

to the tetrahydropyran-2-yl ester (A027), followed by (2) reaction of this derivative with 7-chloro-4-hydroxy-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (A015) to produce compound A028 [i.e., the tetrahydropyran-2-yl ester of 4-(10-carboxydecyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione] and (3) treatment of the latter compound with refluxing acetic acid-benzene, cleaving the ester linkage and yielding dihydropyran and compound A029 [4-(10-carboxydecyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione].

Protection of the carboxyl group of the ω-bromocarboxylic acids was required to prevent its reaction in the subsequent synthetic step. While other methods are available for protecting carboxyl groups,[31-32] the preparation of the tetrahydropyran-2-yl ester[31,33] is particularly suitable since this type of derivative yields the corresponding carboxylic acid by thermal decomposition under relatively mild conditions.

The specific reaction conditions employed in the conversion of the tetrahydropyran-2-yl esters to the corresponding carboxylic acids were determined empirically. We have found it necessary, in order to obtain optimum yields of our products, to reflux a solution containing 0.019 mole of the tetrahydropyran-2-yl esters and 0.122 mole of glacial acetic

acid in 100 ml. of benzene for a period of 16 hours. We found that subsequent removal of all of the acetic acid by azeotropic distillation with excess of benzene proved far better than the alternate neutralization with sodium bicarbonate.

The characterization and confirmation of the chemical constitution of our synthetic compounds were based upon their own chemical analyses, and evaluation of their own infrared and ultraviolet spectra, as well as the chemical analyses and spectral data of compounds constituting derivative or antecedent structures relative to each respective target compound.*

We found it particularly significant that the infrared spectra of the tetrahydropyran-2-yl esters A027, A031 and A034 reflected the same absorption characteristics. When compared with the spectra of the corresponding acids (Y003, Y004 and Y005 respectively), evidence for the presence of the ester linkage in the tetrahydropyranyl derivatives was available in the absence of the carboxyl-OH band (3.8 μ), obvious in the spectra of the acids,[34a] and in the occurrence of the carbonyl band at a wavelength lower than that in the

*The reader is referred to the Appendix for reproductions of the infrared and ultraviolet spectra of our synthetic derivatives, and pertinent related compounds.

spectra of the acids.[34b] In addition, the presence of a series of bands in the 8.7 - 9.7 μ region (i.e., at 8.94, 9.38, 9.50, 9.67, 9.77 μ) appears to be characteristic of this series of tetrahydropyran-2-yl esters. While we have not made any specific assignments for these absorptions, this region is generally associated with the presence of ether-type linkages.[34c]

The same applies to the tetrahydropyran-2-yl esters A028, A032 and A035. Here, too, the carboxyl-OH absorption, registered at 3.8, 4.0 μ in the spectra of the acids, was absent;[34a] the spectra also possessed several bands (at 9.69 and 9.80 μ) which did not occur in that of the corresponding acids, and which may be associated with the presence of C-O-C linkages.[34c]

EXPERIMENTAL SECTION*†

7-Chloro-4-hydroxy-6,2'-dimethoxy-6'-methylgris-2'-ene-

*Melting points were determined with a Swissco melting point apparatus containing silicone fluid and are corrected. Boiling points are uncorrected. Ultraviolet spectra and infrared spectra were obtained, respectively, with the Perkin-Elmer Model 202 and 137B spectrophotometers. A Rudolph Model 62 polarimeter was employed to determine optical rotations. Analyses were performed by Drs. G. Weiler and F. B. Strauss, Oxford, England.

†We gratefully acknowledge the co-operation of Ayerst Laboratories in furnishing us with a generous supply of griseofulvin.

3,4'-dione (A015) was prepared by the method of Arkley and co-workers[32] and, as previously described,[2] it was dried at 100° (2 mm.) for 3 hr., prior to use.

Tetrahydropyran-2-yl 11-Bromoundecanoate (A027).- To a solution of 50.5 g. (0.600 mole) of freshly distilled 3,4-dihydropyran (b.p. 85-86°) and 2 drops of concentrated sulfuric acid in 200 ml. of sodium-dried benzene, a solution of 106.1 g. (0.400 mole) of 11-bromoundecanoic acid (Y003) in 150 ml. of sodium-dried benzene was added slowly (1 hr.), with stirring, at room temperature. The addition funnel was rinsed with 50 ml. of sodium-dried benzene, and this was added to the reaction mixture. The mixture was subsequently stirred for a total of 14 hr. at room temperature in addition to having been left standing, without stirring, for a period of 14.5 hr. Potassium hydroxide (32 g., 0.570 mole) was added to the reaction mixture, and after stirring for 1 hr., a clear solution was obtained by decantation from the insoluble material and subsequent filtration through a layer of Celite. Solvent and excess dihydropyran were removed under reduced pressure, and a mobile, yellow liquid (106.1 g., 75.9%) was obtained; $\lambda_{max.}^{CHCl_3}$ 5.75 μ (ester C=O).

This material was used without further purification in the preparation of compound A028.

Tetrahydropyran-2-yl Ester of 4-(10-Carboxydecyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (A028).- To a mixture of 30 g. (0.217 mole) of anhydrous potassium carbonate and 28.3 g. (0.084 mole) of anhydrous 7-chloro-4-hydroxy-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (A015) in 125 ml. of dimethylformamide, a solution of 32.1 g. (0.092 mole) of tetrahydropyran-2-yl 11-bromoundecanoate (A027) in 40 ml. of dimethylformamide was added slowly (55 min.), with stirring, at room temperature. The addition funnel was rinsed with 15 ml. of dimethylformamide, and this was added to the reaction mixture. The mixture was stirred at room temperature for 1 hr., and at 70° for 24 hr. It was then filtered while still warm, yielding a residue which gave a positive test for bromide ion. The filtrate was treated with 100 ml. of cold, distilled water. The supernatent liquid was decanted from the resulting oily product, which was then washed with two 50-ml. portions of distilled water. The crude product was dissolved in a total of 500 ml. of sodium-dried benzene, and the solution dried over anhydrous sodium sulfate. The benzene solution was decanted from the drying agent and filtered through a layer of Celite. Solvent was removed by distillation under reduced pressure, and a light brown solid (47.7 g., 94.1%) was obtained after drying in vacuo. Recrystallization from ligroine (b.p. 66-75°) gave a white solid product: m.p. 89.3-90.8°; $[\alpha]_D^{27.5}$ + 200.32° (c 1.25, acetone);

$\lambda_{max.}^{EtOH}$ 219 mμ (ε 24,742), 235 (23,983), 291.5 (23,588), 327.5 (6,072); $\lambda_{max.}^{CHCl_3}$ 5.85 μ (C=O), 6.03 (COC=C).

Anal. Calcd. for $C_{32}H_{43}ClO_9$: C, 63.30; H, 7.14; Cl, 5.84. Found: C, 63.07; H, 6.96; Cl, 6.04.

4-(10-Carboxydecyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (A029). Method A.- A solution of 18.9 g. (0.031 mole) of the tetrahydropyran-2-yl ester of 4-(10-carboxydecyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (A028) and 5 ml. (0.087 mole) of glacial acetic acid in 110 ml. of sodium-dried benzene was refluxed for 4 hr. The solution was cooled and a total of 200 ml. of 10% sodium bicarbonate solution was added slowly in portions. Toward the end of the addition, a fine white precipitate resulted which could not be obtained by filtration. The mixture was then acidified with 15% hydrochloric acid and extracted with benzene. The benzene solution was dried over anhydrous sodium sulfate, decanted from the drying agent, and filtered through Celite. Solvent was removed under reduced pressure, and a solid material (12.1 g., 74.7%) was obtained after drying in vacuo. Recrystallization from benzene-ligroine gave white crystals: m.p. 124.2-125.1°; $[\alpha]_D^{24.5}$ + 232.60° (c 1.25, acetone); $\lambda_{max.}^{EtOH}$ 219 mμ (ε 24,818), 235.5 (24,191), 292.5 (23,537), 328 (6,146); $\lambda_{max.}^{CHCl_3}$ 3.95, 4.05 μ (carboxyl-OH),

5.83 (C=O), 6.02 (COC=C).

Anal. Calcd. for $C_{27}H_{35}ClO_8$: C, 62.00; H, 6.75; Cl, 6.78. Found: C, 61.95; H, 6.68; Cl, 6.85.

Method B.- A solution of 40.6 g. (0.067 mole) of the tetrahydropyran-2-yl ester of 4-(10-carboxydecyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (A028) and 6 ml. (0.105 mole) of glacial acetic acid in 110 ml. of sodium-dried benzene was refluxed for 16 hr. The acetic acid was removed by azeotropic distillation with a total of 1.9 l. of sodium-dried benzene, yielding 42.1 g. of crude product after drying in vacuo. This material was divided into three portions and each portion was extracted with 1.0 l. of refluxing ligroine for 2 hr. The insoluble material remaining after decantation of the ligroine was recrystallized from benzene-ligroine to give 0.71 g. of product, m.p. 123.3-124.2°.

Crude material recovered from the recrystallization mother liquors, combined with that obtained from the ligroine extractions, totaled 23 g. This was divided into two portions, each of which was dissolved in 100 ml. of sodium-dried benzene containing 7 ml. (0.122 mole) of glacial acetic acid, and the solutions refluxed for 16 hr. The acetic acid was removed by azeotropic distillation and the residual dried products

extracted with 500 ml. refluxing ligroine for 2 hr. The ligroine was decanted and the resulting total solid material (18.0 g.) was recrystallized from benzene-ligroine to give 4.30 g. of product with m.p. 119.0-120.7°. The infrared spectrum of a chloroform solution of this substance was superimposable on that of the analytical sample obtained by Method A.

Tetrahydropyran-2-yl 10-Bromodecanoate (A031) was prepared from 14.1 g. (0.168 mole) of 3,4-dihydropyran and 27.9 g. (0.111 mole) of 10-bromodecanoic acid (Y004) by the procedure described for the synthesis of compound A027. The product (27.8 g., 74.7%) was a mobile, yellow liquid; $\lambda_{max.}^{CHCl_3}$ 5.80 μ (ester C=O).

This material was used without further purification in the preparation of compound A032.

Tetrahydropyran-2-yl Ester of 4-(9-Carboxynonyl-1-oxy)-7-chloro 6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (A032).- This compound was prepared from 22.8 g. (0.067 mole) of anhydrous 7-chloro-4-hydroxy-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (A015), 24.8 g. (0.074 mole) of tetrahydropyran-2-yl 10-bromodecanoate (A031) and 25.0 g. (0.181 mole) of anhydrous potassium carbonate by the procedure described for

the preparation of compound A028. The crude product (39.3 g., 98.3%), recrystallized from ligroine (b.p. 66-75°), gave a white powder: m.p. 96.3-98.0°; $[\alpha]_D^{27.5}$ + 207.20° (c 1.25, acetone); $\lambda_{max.}^{EtOH}$ 217 mμ (ε 25,741), 233 (24,970), 291 (24,881), 330 (6,524); $\lambda_{max.}^{CHCl_3}$ 5.84 μ (C=O), 6.04 (COC=C).

Anal. Calcd. for $C_{31}H_{41}ClO_9$: C, 62.78; H, 6.97; Cl, 5.98. Found: C, 62.67; H, 6.99; Cl, 6.08.

4-(9-Carboxynonyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (A033).- A solution of 21.9 g. (0.037 mole) of the tetrahydropyran-2-yl ester of 4-(9-carboxynonyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (A032) in 200 ml. of sodium-dried benzene was divided into two portions, each of which was treated with 7 ml. (0.122 mole) of glacial acetic acid and refluxed for 16 hr. For each portion, the acetic acid was removed by azeotropic distillation with a total of 1.5 l. of sodium-dried benzene. After the residues were dried in vacuo, each was extracted with 1.0 l. of refluxing ligroine for 2 hr. The insoluble material (total 14.3 g., 76.1%) remaining after decantation of the hot ligroine was recrystallized from benzene-ligroine, yielding 3.8 g. of a white powder: m.p. 123.5-137.7°; $[\alpha]_D^{27.5}$ + 224.60° (c 1.25, acetone); $\lambda_{max.}^{EtOH}$ 217.5 mμ (ε 23,262), 234.5 (22,727), 291 (22,524), 327.5 (5,981); $\lambda_{max.}^{CHCl_3}$ 3.84 μ

(carboxyl-OH), 5.89 (C=O), 6.07 (COC=C).

Anal. Calcd. for $C_{26}H_{33}ClO_8$: C, 61.35; H, 6.54; Cl, 6.97. Found: C, 61.51; H, 6.60; Cl, 6.84.

Tetrahydropyran-2-yl 8-Bromooctanoate (A034) was prepared from 13.5 g. (0.160 mole) of 3,4-dihydropyran and 24.0 g. (0.108 mole) of 8-bromooctanoic acid (Y005) by the procedure described for the synthesis of compound A027. The product (23.6 g., 71.4%) was a mobile, yellow liquid; $\lambda_{max.}^{CHCl_3}$ 5.77 μ (ester C=O).

This material was used without further purification in the preparation of compound A035.

Tetrahydropyran-2-yl Ester of 4-(7-Carboxyheptyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (A035).- This compound was prepared from 22.6 g. (0.067 mole) of anhydrous 7-chloro-4-hydroxy-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (A015), 22.2 g. (0.072 mole) of tetrahydropyran-2-yl 8-bromooctanoate (A034) and 25.0 g. (0.181 mole) of anhydrous potassium carbonate by the procedure described for the preparation of compound A028. The crude product (27.6 g., 73.2%), recrystallized from ligroine, gave a white powder: in melting point determinations, the compound softened

at 86.3° and gradually yielded a clear melt at 104.4°; $[\alpha]_D^{27.5}$ + 207.20° (c 1.25, acetone); $\lambda_{max.}^{EtOH}$ 217.5 mμ (ε 24,485), 234 (24,750), 291.5 (24,580), 330 (6,272); $\lambda_{max.}^{CHCl_3}$ 5.87 μ (C=O), 6.07 (COC=C).

Anal. Calcd. for $C_{29}H_{37}ClO_9$: C, 61.64; H, 6.60; Cl, 6.27. Found: C, 61.70; H, 6.70; Cl, 6.32.

4-(7-Carboxyheptyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (A036) was prepared from 20.0 g. (0.035 mole) of the tetrahydropyran-2-yl ester of 4-(7-carboxyheptyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (A035) by the procedure described for the preparation of compound A033. The crude product (16.3 g., 97.0%), upon recrystallization from benzene-ligroine, gave a white powder: m.p. 193.7-196.0°; $[\alpha]_D^{28.5}$ + 244.00° (c 1.25, acetone); $\lambda_{max.}^{EtOH}$ 218.5 mμ (ε 24,024), 233.5 (23,254), 290.5 (23,206), 328 (5,964); $\lambda_{max.}^{CHCl_3}$ 3.83, 4.03 μ (carboxyl-OH), 5.84 (C=O), 6.04 (COC=C).

Anal. Calcd. for $C_{24}H_{29}ClO_8$: C, 59.94; H, 6.08; Cl, 7.37. Found: C, 59.92; H, 6.22; Cl, 7.24.

PART THREE

INTERACTIONS OF OUR COMPOUNDS WITH PURIFIED SKIN CONSTITUENTS

As indicated in Part One of This Report, we are employing monomolecular-layer techniques to assist in the elucidation of those chemical and physical characteristics essential for anchoring to or localization in dermal tissue of topically applied, or systemically administered, potential insect-repellents.

Prior to the actual evaluation of our compounds, it was necessary to incorporate several significant modifications in the instrumentation we previously described.[1] These included improved Teflon coating of our troughs as well as development of a new compression barrier and end-piece system. The changes in our instrumentation were made principally to remedy difficulties experienced in the evaluation of our compounds in comparatively complex systems, and to permit their evaluation under uniform conditions.

We tested the effectiveness of our newly developed systems by evaluating films of purified specimens of stearic acid and cholesterol spread (a) on redistilled water, as well as (b) on an aqueous griseofulvin solution (see Figures 7 and 8). In this regard, it is significant that the pressure-area curves both for

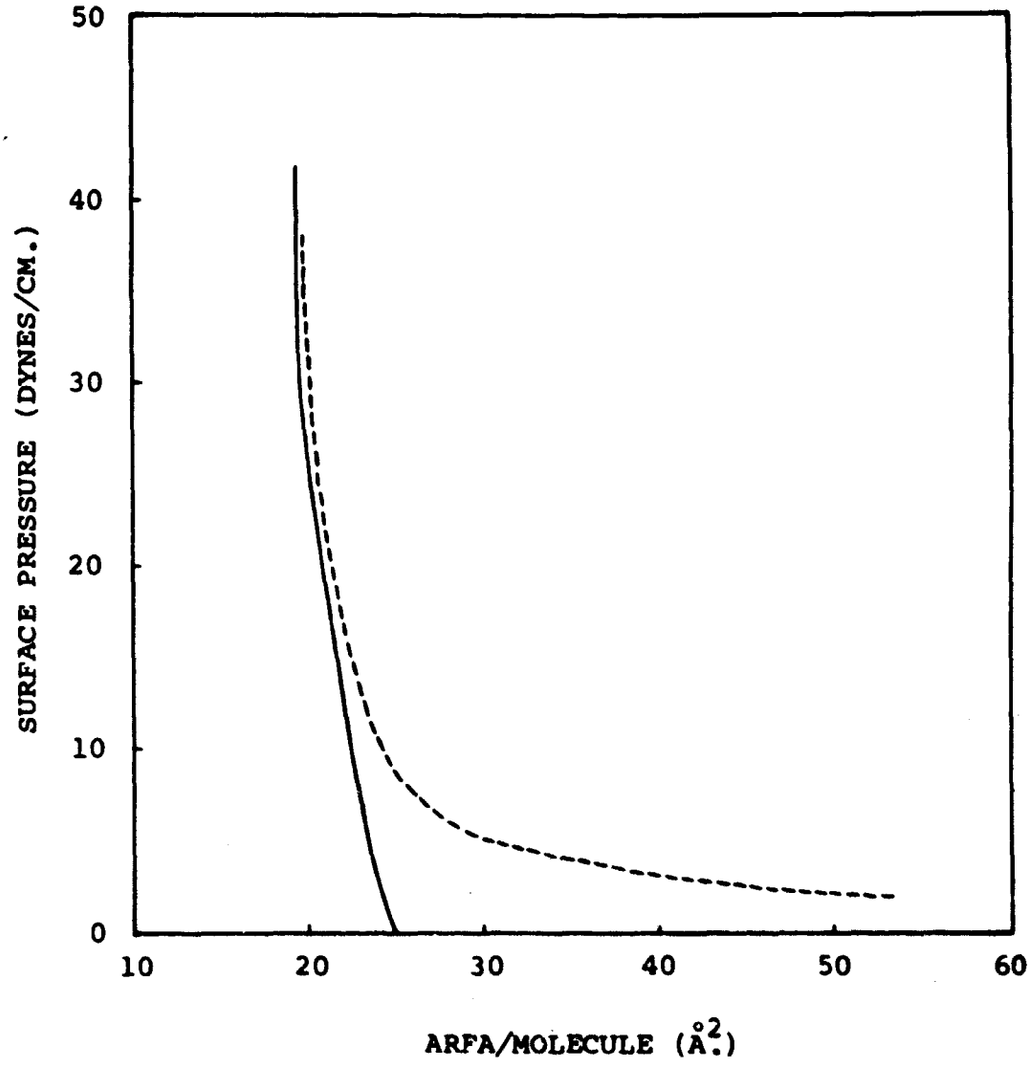


Fig. 7.— Surface pressure vs. area per molecule for stearic acid spread on: redistilled water (——); 5.7 X 10^{-5}M solution of griseofulvin in redistilled water (---). In the latter case the corrected surface pressure is shown.

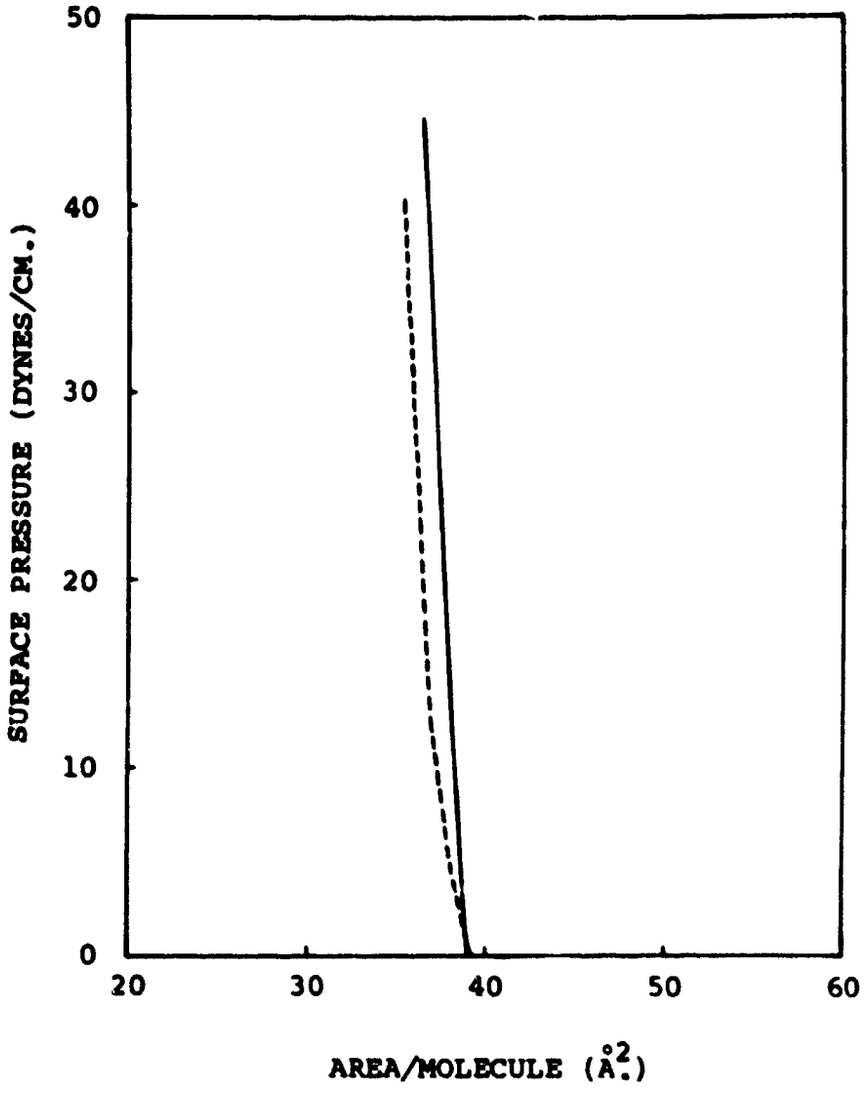


Fig. 8.— Surface pressure vs. area per molecule for cholesterol spread on: redistilled water (——); 5.7 X 10^{-5}M solution of griseofulvin in redistilled water (---). In the latter case the corrected surface pressure is shown.

stearic acid and for cholesterol spread on water agree well with those reported by others,[35-36] using similar instrumentation and methods.

We also found particularly interesting the pressure-area isotherms we obtained when these same film-forming materials were spread on the substrate containing griseofulvin.

The pressure-area curve for stearic acid spread on this substrate indicates that griseofulvin was present in the monolayer, and that, as the film pressure was increased, most of it was forced out of the film (i.e., the curve approached that of pure stearic acid). It should be also noted that the collapse pressure for this curve was slightly lower than that for pure stearic acid. This appears to be consistent with the observation[15] that the presence of irregularly shaped molecules in films of regularly oriented molecules renders the film less stable.

The curve for cholesterol spread on the solution of griseofulvin is interesting in that it is displaced slightly toward a lower area per molecule compared with that spread on redistilled water. This suggests that griseofulvin either facilitates a closer packing of the cholesterol molecules than can be obtained in the film of the pure substance or, more likely, that the gri-

seofulvin causes some of the cholesterol molecules to leave the area-determining position in the surface due to a process of "interfacial dissolution."[15] In this regard it is interesting to note that the solubility of griseofulvin in aqueous media has been increased by the presence of steroid-type molecules (i.e., bile acid salts).[37]

Our results, demonstrating the effects that griseofulvin has upon the known skin constituents, stearic acid and cholesterol, seem to corroborate other evidence[38-39] that the presence of lipid material in skin tissue may be associated with increased uptake of griseofulvin.

Moreover, in view of observations by others[40] indicating that certain polyene antibiotics exert their effects by influencing the orientation of sterol molecules in the cell membranes of sensitive organisms, griseofulvin's antidermatophytic effects might be associated with its action upon lipid cell wall material of susceptible dermatophytes.

Initially, we anticipated studying all derivatives of our synthetic series by the same method as the one we employed in our studies on griseofulvin. The extremely poor solubility of some of these compounds in water, however, precluded their evaluation by this technique.

In view of the good solubility, in chloroform, of most of our synthetic compounds, as well as that of the lipophilic skin constituents we expected to use, we developed a method for evaluating our compounds in mixed monomolecular films. The results of our experimental work with mixed films of stearic acid and compounds A013, A014 and A029, along with appropriate control data for stearic acid alone, are shown in Figure 9.* It is most gratifying to note that, after many months of hard work, we have developed a procedure of dependable accuracy and remarkable reproducibility, considering the complex systems with which we are dealing.

While we cannot offer a completely rigorous interpretation of the significance of the results until we obtain data on other members of our series of derivatives, the results shown do illustrate the effectiveness of the method for distinguishing characteristics among the compounds evaluated. For example, it may be seen that both compounds A014 and A029 have a greater affinity for the stearic acid monolayer than does compound A013. Further, it appears that somewhat more of compound A029 is retained in the film at higher pressures compared with compound A014, even though, at lower pressures, much higher area-per-

*Each curve represents three independent determinations. The reader is also referred to the Appendix for the pressure-area curves for each mixed film system studied.

molecule values are observed in the latter instance.

We are in the process of examining the pressure-area curves for the pure test compounds alone. This will permit us to examine more accurately the nature of the association of our synthetic entities with the skin component of the monolayer.

In a system with an ideally additive behavior, which exists when the molecular arrangement in the mixed monomolecular layer is not affected by forces like van der Waals', other molecular association, etc., equation (1)[41] may be employed:

$$A_T = n_C A_C + n_S A_S \quad (1)$$

where A_T is the total area of the mixed film, n_C is the number of molecules of the pure test compound, and n_S is the number of molecules of the pure skin component, while A_C and A_S are the molecular areas of the test compound and skin component, respectively, in pure films of these materials, at the same surface pressure.

The comparison of experimentally determined isotherms, in a given mixed film system, with theoretical pressure-area curves, derived from the above equation, should make evident any deviations from ideal behavior in terms of concepts reasonably well established in contemporary chemistry.

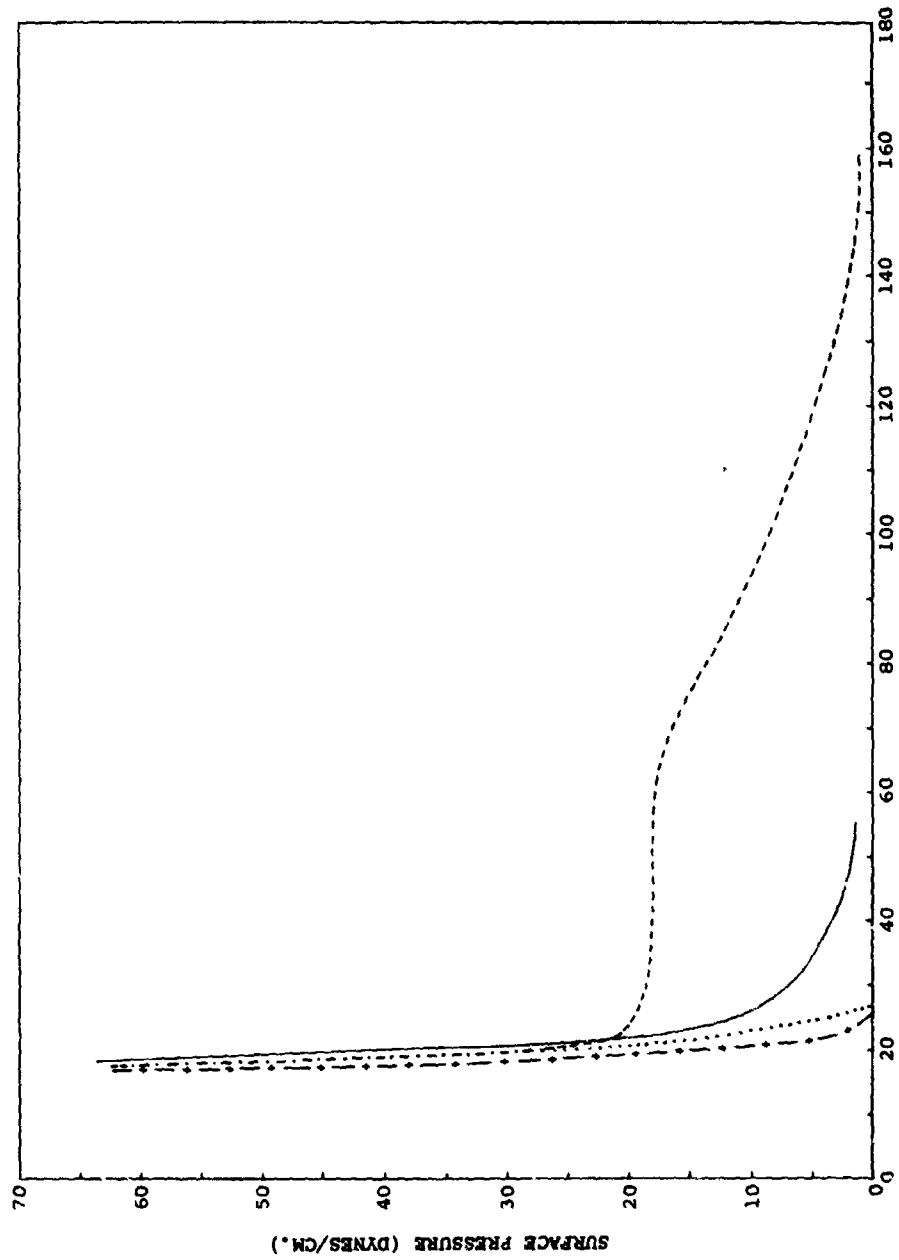


Fig. 9.— Pressure-area curves for stearic acid (-·-), an equimolar mixture of stearic acid and compound A013 (····), an equimolar mixture of stearic acid and compound A014 (---) and an equimolar mixture of stearic acid and compound A029 (——). At 27.5 dynes/cm., the ascending curve (-·-) represents the merged isotherms for the runs with A013 and A014.

EXPERIMENTAL SECTION

Materials*.- The film-forming materials we employed were high-purity substances. Stearic acid (m.p. 71.1-72.0°), prepared by saponification of fractionally distilled methyl stearate, was obtained in purity greater than 99%.† The cholesterol utilized was procured chromatographically pure (99+ %) (Sigma Chemical Company); it melted at 148.8-149.4°.

The chemistry and properties of the compounds evaluated (i.e., compounds A013, A014 and A029‡) by mixed monolayer techniques were previously described.[1-2] All of these compounds were of analytically pure grade.

Griseofulvin, supplied by Ayerst Laboratories, was recrystallized once from benzene, yielding white needles: m.p. 220.5-221.4°; $[\alpha]_D^{26}$ + 331.03° (c 1.015, acetone); $\lambda_{max.}^{EtOH}$ 215 mμ (ε 23,107), 235 (22,754), 292 (23,636), 330 (5,644); $\lambda_{max.}^{CHCl_3}$

*Melting points were determined with a Swissco melting point apparatus containing silicone fluid and are corrected. Ultraviolet spectra and infrared spectra were obtained, respectively, with the Perkin-Elmer Model 202 and 137B spectrophotometers. A Rudolph Model 62 polarimeter was employed to determine optical rotations. Analyses were performed by Drs. G. Weiler and F. B. Strauss, Oxford, England.

†We obtained this material through the courtesy of R. J. Grabbenstetter, Research and Development Department, The Procter and Gamble Company.

‡For compound A029, see Part Two of This Report.

5.85 μ (C=O), 6.02 (COC=C). These properties were consistent with those reported in the literature.[42-43]

Anal. Calcd. for $C_{17}H_{17}ClO_6$: C, 57.88; H, 4.86; Cl, 10.05. Found: C, 57.79; H, 4.78; Cl, 10.30.

The substrate water was laboratory distilled water redistilled through a 45-cm. Vigreaux column from aqueous permanganate. It had a pH of 6.0, and gave a value of 0.15 p.p.m. in a conductometric measurement of ionizable substances expressed as sodium chloride.* The corrected surface tension of water obtained by this method fell within the range already reported (i.e., 70.26-70.87 dynes/cm.).[44]

Solutions of griseofulvin (20 μg./ml.; 5.7×10^{-5}M) were prepared[39] by boiling the substrate water to which griseofulvin had been added, followed by cooling before transferring and bringing the solution to volume in a volumetric flask. The absorbance at 295 mμ[39] for the solutions prepared in this manner was 1.35 ± 0.02. Before being employed in the corresponding control experiments, substrate water was treated in an identical manner to that used in the preparation of the

*The conductometric measurement was made by Doctor Kenneth E. Avis, Department of Pharmaceutics, University of Tennessee College of Pharmacy.

griseofulvin solutions.

The spreading solvents utilized were spectro-grade benzene and chloroform. Examination of these solvents for the presence of surface-active contaminants was performed by depositing suitable amounts of the solvent on the surface of substrate water and, after evaporation of the solvent had occurred, compressing the barrier toward the float. The occurrence of surface pressure, indicative of the presence of nonvolatile impurities, was not observed in these tests.

Apparatus.- Our surface balance is a refinement* of the commercially available Hydrophil Balance (Central Scientific Company) (see Figures 10 and 11). While, in our initial work, only the interior of the trough was Teflon coated, we now employ troughs completely coated with this material.† The compression barrier consists of two parts. That part (B, Figure 10) which comes into contact with the monomolecular film is a

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*We wish to acknowledge the work of Mr. W. A. Frase of the Instrument Division, University of Tennessee Medical Units, in constructing most of the modifications described. Mr. B. Carpenter, Memphis, Tennessee, fashioned the Teflon parts of our currently employed end-piece system.

†Our troughs were coated by the Slipmate Company, Franklin Park, Illinois. The coating consisted of a pigmented TFE Teflon basecoat (0.001-0.0015 in. thick) and an unpigmented TFE Teflon topcoat (0.001-0.0015 in. thick) applied over a pigmented primer (0.005 in. thick).

piece of solid Teflon (19.8 cm. x 3.7 cm. x 0.6 cm.) to which is screwed* a heavy brass bar (19.8 cm. x 3.7 cm. x 1.9 cm.). The bar carries an aluminum pointer (D, Figure 10) which rides along the metric scale attached to the side of the trough and indicates the position of the front edge of the barrier. The second part (A, Figure 10) of the compression barrier also consists of a piece of solid Teflon (20.7 cm. x 3.6 cm. x 0.6 cm.) secured by screws to a lighter brass bar (20.7 cm. x 3.6 cm. x 0.8 cm.). This part is attached to the edges of the trough by means of an adjustable Teflon sping-pressure clamp, and when it is connected to the pin emanating from the operating drive-screw, it serves in moving part B toward the float. Part A has a restraining arm (C, Figure 10) on each side permitting only slight lateral movement (2 mm.) of part B.

Our float (C, Figure 11) and end-piece systems (A, Figure 11) are also constructed of Teflon and are patterned after those described by Mann and Hansen.[45] The solid Teflon float (12.0 cm. x 0.6 cm. x 0.1 cm.) is fastened to the Teflon end-piece system by means of thin Teflon foils (B, Figure 11) inserted into slits cut in the float and end pieces. The float is connected to the torsion wire of the film balance via a

*The screws do not pierce any portion of the Teflon which comes into contact with the substrate.

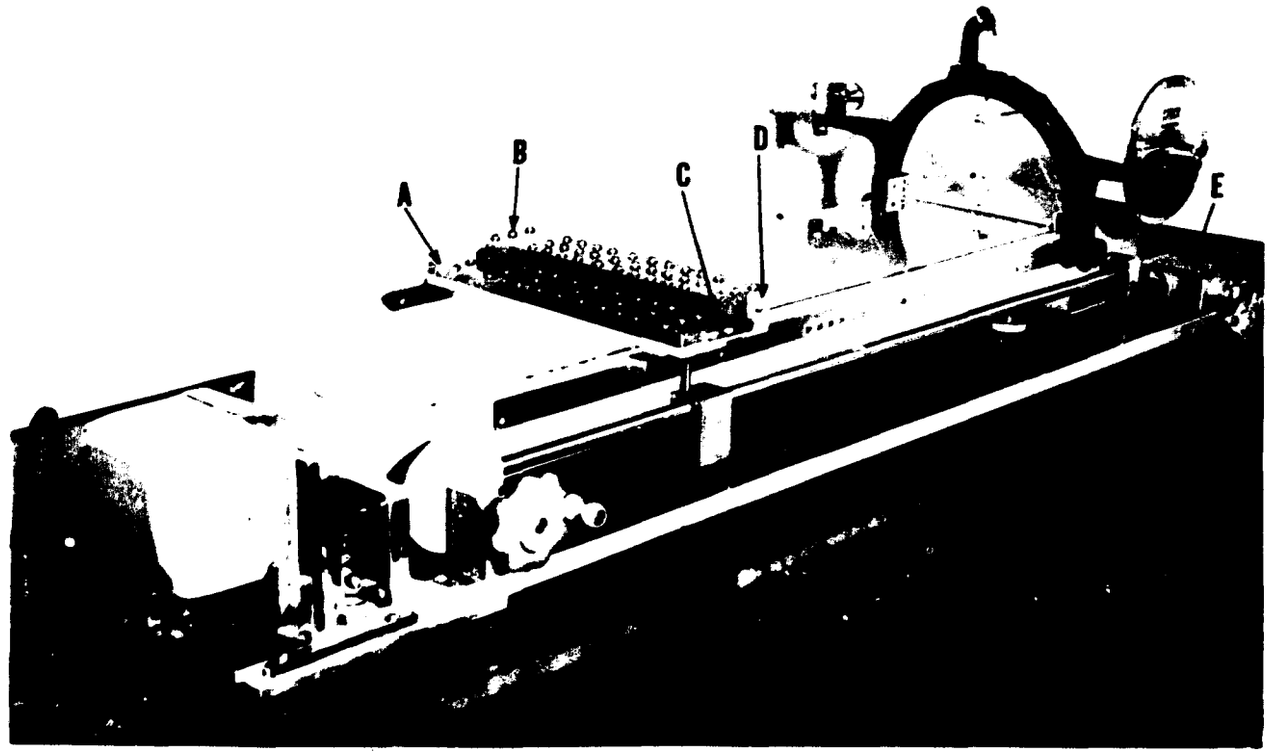


Fig. 10.— Our modification of surface balance employed in our monolayer studies. (See text for explanation of parts indicated.)

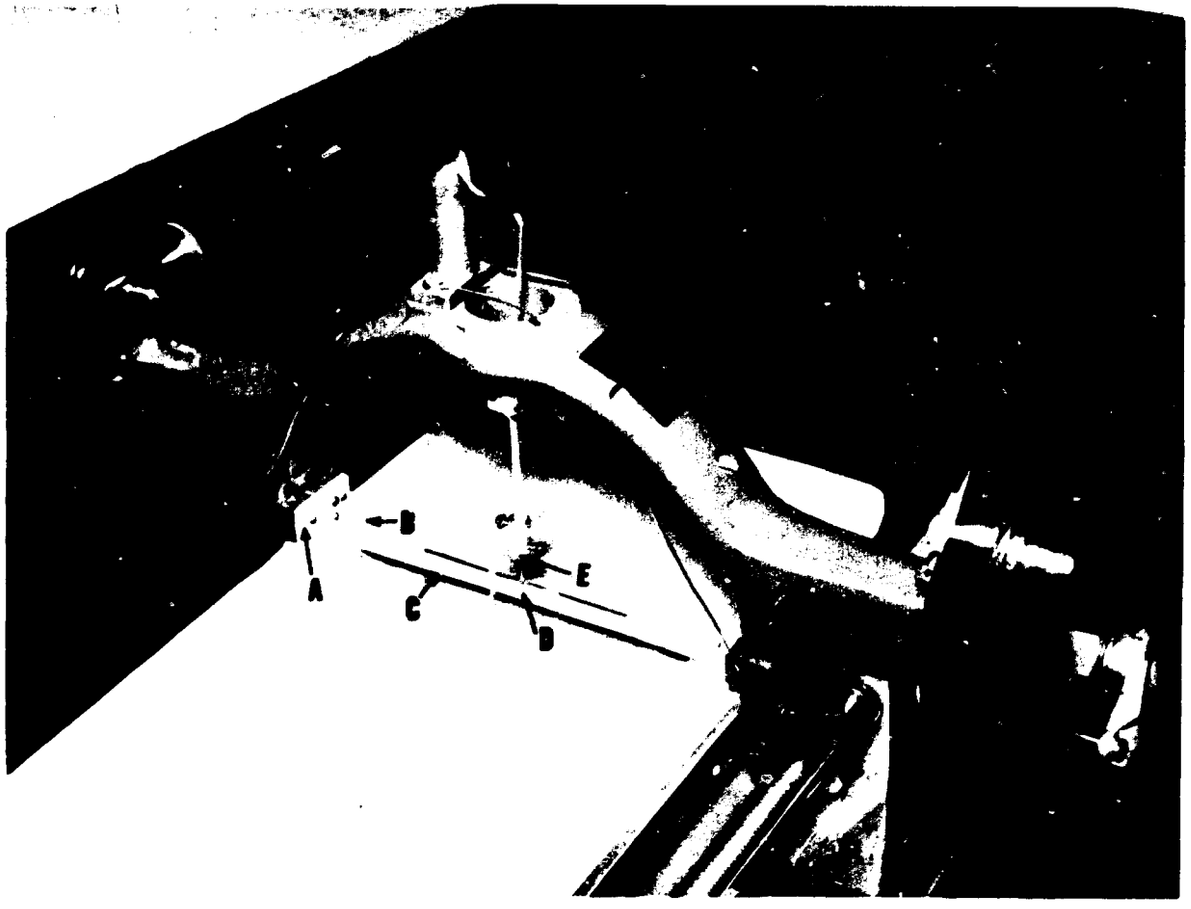


Fig. 11.— Float and end-piece units used in our modification of the surface balance. (See text for explanation of parts indicated.)

three-prong, platinum-wire fork (D, Figure 11) which protrudes through slightly oversize holes drilled into the float, and which is secured to the vertical arm attached to the torsion wire by the connecting sleeve E (Figure 11).

The leveling screws on the trough are set into corresponding cavities drilled in an aluminum baseplate (81.5 cm. x 22.0 cm. x 0.7 cm.) provided with its own leveling screws, and with a barrier-transport mechanism for either manual or motor-driven operation. (In our experimental work, we normally employed a motor which would drive the barrier at a speed of 0.1 mm./sec.). The baseplate also carries a switch (E, Figure 10) at the float-end of the trough which may be used to prevent accidental damage to the motor or float by stopping movement of the barrier at a predetermined distance from the float.

Procedures.- The torsion-balance wire was calibrated with class M analytical weights, and the calibration checked at appropriate periodic intervals.

The trough and all Teflon parts of our apparatus which come into contact with the substrate and monolayer are thoroughly cleaned by soaking in reagent-grade benzene followed by rinsing with spectro-grade benzene and redistilled water.

All glassware is scrupulously cleaned with hot chromic acid cleaning solution after thorough rinsing with reagent-grade benzene, 2-butanone and redistilled water. Following this treatmant, the glassware is repeatedly rinsed with distilled and redistilled water, and is then dried.

The cleaned equipment is assembled and utilized within an isolation cabinet (Kewaunee Scientific Equipment) permitting operation in a dust-free and draft-free environment.

In some experiments it was necessary to coat the junction between our end-piece system and the trough edge. We applied a small amount of melted paraffin (m.p. 60-62°) for this purpose.[46]

All of our experiments were carried out at room temperature in an air-conditioned laboratory. While the temperature of the substrate, determined at periodic intervals throughout the experiment, was normally 24 ± 2°, it rarely varied more than 1° in a given experiment.

In our experiments involving the effect of griseofulvin upon monomolecular layers of stearic acid and cholesterol, we employed the following procedure. The trough

was filled with the substrate water or solution to a level slightly above the edges, and the surface in front of , and behind, the float was swept thoroughly with Teflon sweeping barriers. The level of the substrate was adjusted properly, and a check was made on the cleanliness of the substrate surface by manually moving a Teflon barrier toward the float. If no surface pressure was detected, the compression barriers were set up such that the initial area was 490 $cm.^2$ to 560 $cm.^2$ The film-forming materials were then applied to the surface of the substrate, by means of an Agla micrometer-syringe (Burroughs Wellcome Co.), in spectro-grade benzene. After a period of 20 min. during which the solvent evaporated, the compression barrier was moved slowly toward the float. Usually 2-mm. decrements were employed starting about 3 cm. before pressure was first observed, and as the pressure increased rapidly with a small decrease in area, 1-mm. decrements were used. In each case, there was a 1-min. interval between the time when the barrier was stopped and that when the reading of surface pressure was made. After the monolayer had collapsed, we checked for leakage with purified talc and/or by observing any deflection of the indicator-needle when a sweeping barrier, placed behind the float, was moved toward it. The apparatus was then cleaned, as previously described, prior to use in a subsequent determination. Each of the pressure-area isotherms (Figures 7 and 8) was obtained by

averaging the results of three independent experiments. In the case of the isotherms for stearic acid or cholesterol spread on the aqueous solution of griseofulvin, the corrected surface pressure[36] is shown. This was determined to permit direct comparison with values obtained when the substrate was redistilled water. The correction factor is actually the lowering of the surface tension of the redistilled water produced by the griseofulvin (i.e., it is the surface pressure exerted by the griseofulvin solution). This was determined to be 0.6 dyne/cm. by use of methods previously described.[44]

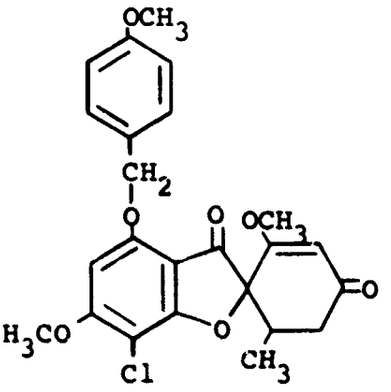
In experiments involving the evaluation of our compounds in mixed monomolecular-layer systems, we employed the following procedure. The trough was filled with the substrate water, and the surface was swept clean and examined for contamination as described previously. The compression barrier was affixed such that the initial area was 560 cm.^{2}, and a solution of stearic acid in spectro-grade chloroform was deposited on the surface of the substrate. The resulting film was compressed continuously, at a rate of 0.5 mm./sec., to the collapse point. During this operation pressure was continuously balanced, but not recorded. The collapsed film was swept from the surface, and this process was repeated. The surface was again swept and checked for contamination in the usual manner. A freshly prepared solution of stearic

acid in spectro-grade chloroform was deposited on the substrate, and after allowing 20 min. for the solvent to evaporate, the compression barrier was moved toward the float in 5-mm. decrements, then 2-mm. decrements (at low pressures) and finally 1-mm. decrements (at higher pressures). As described previously, a 1-min. interval was employed between each decrement. After the film collapsed, it was thoroughly swept from the surface, and again a check was made for the absence of surface contamination. Then an equimolar mixture of the test compound and stearic acid in spectro-grade chloroform was deposited on the substrate, and after the solvent evaporated (20 min.), the film was compressed as described above for stearic acid alone. Following film-collapse, the trough was cleaned prior to the next experiment. Each of the pressure-area isotherms illustrated (Figure 9) for the 1:1 mixtures of our compounds with stearic acid, as well as for the stearic acid control, is derived from three independent determinations.

PART FOUR

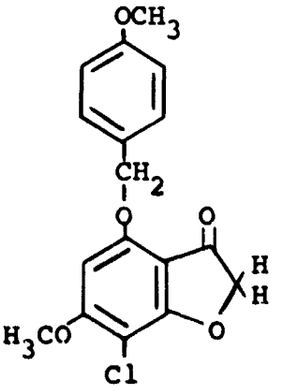
A. SYNTHESIS OF ADDITIONAL QUANTITIES OF PREVIOUSLY REPORTED COMPOUNDS FOR THE EVALUATION OF THEIR INSECT-REPELLENT AND ANTIDERMATOPHYTIC EFFICACY

A very substantial amount of time was spent on the preparation of additional quantities of four, previously reported[1] compounds; i.e., compounds A003, A014, A016 and A018 (see structures and nomenclature in Table I). Additional quantities of compounds A013 and A017 (see structures and nomenclature below) had been already prepared earlier. This became



A013

7-Chloro-6,2'-dimethoxy-4-(p-methoxybenzyloxy)-6'-methylgris-2'-ene-3,4'-dione



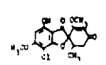
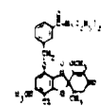
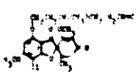
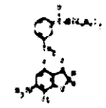
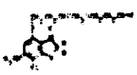
A017

7-Chloro-6-methoxy-4-(p-methoxybenzyloxy)-coumaran-3-one

necessary, firstly, in order to reevaluate the insect-repellent properties of our compounds using the standard method

Table I

Data on Compounds Re-synthesized

Compound Code	Structure	Chemical Nomenclature	Additional Total Quantity Synthesized, Grams	Number of Independent Synthetic Runs	Yield, %[a]	Method(s) Employed for Confirmation of Chemical Constitution	Reference to Preparative Procedure
A005		3,5-Dimethoxyphenol	320.5	3	...[b]	Boiling Point	43
A006		2-Chloro-3,5-dimethoxyphenol	180.0	4	47.4	Melting Point	43
A007		2-Hydroxy-3,6-dichloro-4,6-dimethoxyacetophenone	85.1	3	33.[illegible]	Melting Point	43
A009		7-Chloro-4,6-dimethoxycoumaran-3-one	74.7	2	Quantitative	Melting Point	43
A004		7-Chloro-4-hydroxy-6-methoxy-coumaran-3-one	19.0	2	55.[illegible]	Melting Point Infrared Spectrum Ultraviolet Spectrum	2
A011		8-Bromo-2,6-dimethyloctene-2 (citronellyl bromide)	29.9	1	-	Boiling Point Refractive Index	2
A015		7-Chloro-4-hydroxy-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione	181.0	5	55.0	Melting Point Infrared Spectrum	2
A003		7-Chloro-4-[3-(N,N-diethylcarbamyl)-benzyloxy]-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione	11.3	1	58.3	Melting Point	2
A014		7-Chloro-4,6'-dimethoxy-6',2'-dimethyl-6-octenyl-[oxy]-6'-methylgris-4'-ene-3,6'-dione	8.1	1	62.4	Melting Point	4
A013		7-Chloro-4-[3-(N,N-diethylcarbamyl)-benzyloxy]-6-methoxycoumaran-3-one	4.2	1	18.6	Melting Point Infrared Spectrum	4
A010		7-Chloro-4-methoxy-6-[3,7-dimethyl-6-octenyl]-oxy]-coumaran-3-one	1.7	1	18.9	Melting Point Infrared Spectrum	4

[a] Calculated for purified product. [b] Since experiments where the number of independent synthetic runs is greater than one, the yield given is an average value. [c] Yield was calculated due to recycling of an intermediary material and unreacted starting material in subsequent runs.

employed by U.S.D.A.'s Entomology Laboratory in Gainesville, Florida, in which some of our compounds yielded inconsistent results.

Secondly, we wanted to provide our compounds in sufficient quantities to permit testing of insect-repellency by other laboratories, as well as by methods which would permit the measurement of even slight differences between the respective repellent activities.

Thirdly, it was our intent to make available sufficient amounts of our derivatives for the evaluation of their dermophilic and antidermatophytic characteristics.

Although we were cognizant of the substantial investment of time the re-syntheses would require, the first of the factors, alone, did not give us any other alternative. For example, the preparation of one of the key intermediates (compound A004) involves a sequence of five synthetic steps.

The compounds were prepared using the procedures previously described.[1-2,43] The data are summarized in Table I.

The results reported by Doctor Carroll N. Smith (Entomology Research Division, U.S.D.A.) and Mr. Philip Kashin (IIT

Research Institute) are summarized in Part Five. The findings of Doctor Robert G. Crounse (Division of Dermatology, Johns Hopkins University School of Medicine) are described in Part Six.

B. REEVALUATION OF THE CHEMICAL AND PHYSICAL PROPERTIES, AND RECONFIRMATION OF THE MOLECULAR CONSTITUTION OF TWO COMPOUNDS YIELDING INCONSISTENT RESULTS IN INSECT-REPELLENT TESTS

The report of Doctor Carroll N. Smith and Jane Roos from U.S.D.A.'s Entomology Laboratory in Gainesville, Florida, dated June 14, 1965, indicated no significant difference between the effectiveness of the prominent standard N,N-diethyl-m-toluamide (ENT-22542) and our compound A013 (ENT-28340) in their respective abilities to repel Aedes aegypti mosquitoes. Our compound A017 (ENT-28343) was reported to exert also potent effects, but was indicated to be somewhat less active than the latter.- A second set of tests, carried out October 1, 1965, failed to confirm these results. Neither were the investigators able to duplicate the original findings, reported June 14, 1965, in subsequent evaluations.

Even though we did not anticipate that our compounds A013

Table II

Physicochemical Properties of Compound A013
(A013:PADBI-61h)

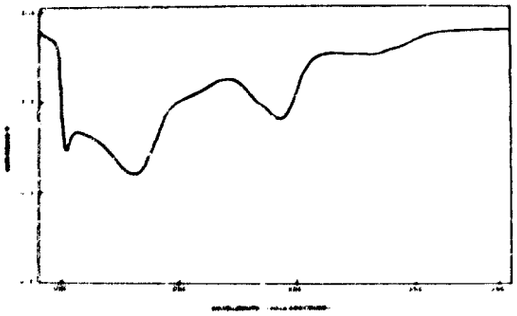
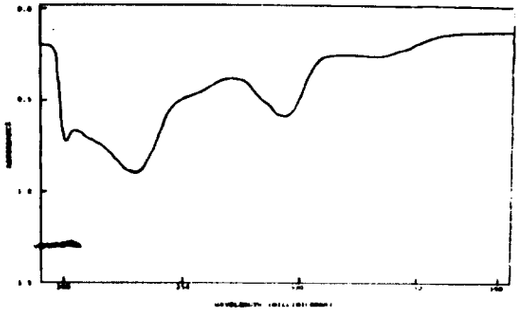
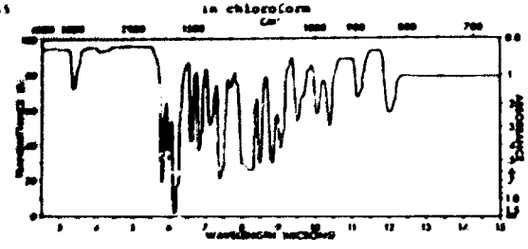
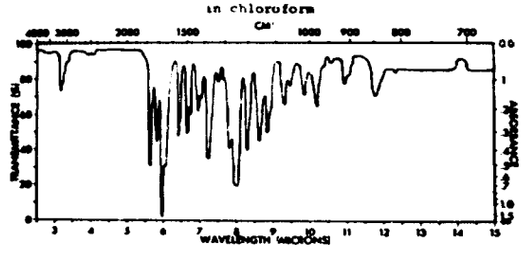
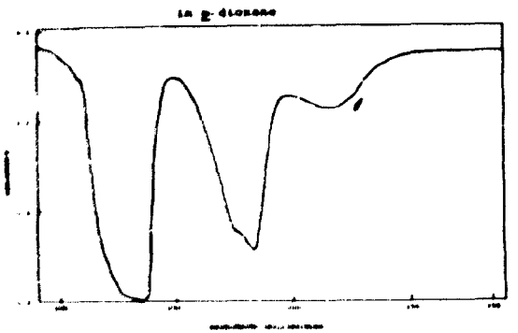
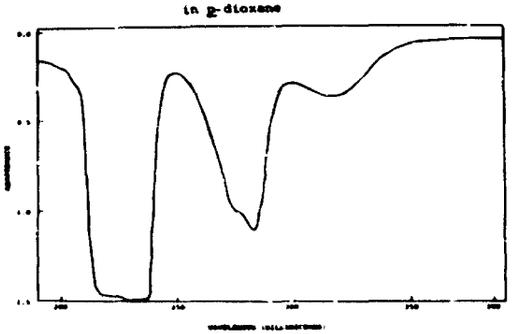
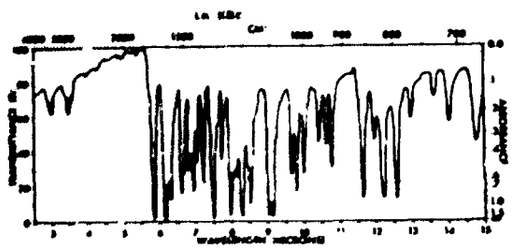
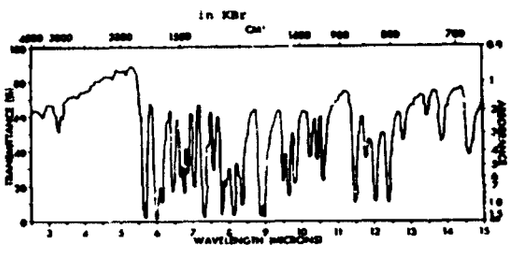
	Date	Original Determination	Recheck	Date
Melting Point	30 Dec. 64	219.0-220.0° dec. (uncorr.)	219.0-221.0° dec. (uncorr.)	12 Nov. 65
Optical Rotation	9 Mar. 65	$[\alpha]_D^{24}$ +246.32° (c 1.224, p-dioxane)	$[\alpha]_D^{26.8}$ +241.83° (c 1.224, p-dioxane)	27 Oct. 65
Ultraviolet Absorption	27 Feb. 65	in 95% EtOH 	in 95% EtOH 	26 Oct. 65
Infrared Absorption	29 Jan. 65	in chloroform 	in chloroform 	29 Oct. 65

Table III

Physicochemical Properties of Compound A017
(A017:PADBI-136c)

	Date	Original Determination	Recheck	Date
Melting Point	4 Nov. 65	191.5-193.0° dec. (uncorr.)	191.5-193.0° dec. (uncorr.)	12 Nov. 65
Optical Rotation		optically inactive	optically inactive	
Ultraviolet Absorption	11 Nov. 65	in p-dioxane 	in p-dioxane 	5 Nov. 65
Infrared Absorption	14 Nov. 65	in KBr 	in KBr 	8 Nov. 65

and A017 could be unstable, under the usual conditions of storage and evaluation, from a strictly scientific point of view, this factor could not be eliminated without appropriate experimental work. In Tables II and III, we summarized the results of our original and recheck determinations for compounds A013 and A017, respectively. Inspection of the data reveals no significant differences,* confirming experimentally that no physical or chemical change occurred in the samples of the materials actually used by the Entomology Laboratory compared to the original entries in our record books.

In addition, we had to provide the other four compounds (A003, A014, A016 and A018) in sufficiently large additional quantities to permit the reevaluation of their respective insect-repellencies; we were compelled to eliminate the possibility that two of the latter could be the potent insect-repellents instead of the reported compounds A013 and A017.

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*For the benefit of those who are not experienced in handling chemical data, slight variations are results of variables such as recorder pen response, uniformity of KBr pellets, minor temperature fluctuations and determinations by different operators, etc.

PART FIVE

SUMMARY OF CURRENTLY AVAILABLE DATA ON THE INSECT-REPELLENT PROPERTIES OF OUR COMPOUNDS

In tests designed by Doctor Carroll N. Smith (Investigations Leader, Entomology Research Division, U.S.D.A.) for the detection of measurable repellent activities as well as slight differences in repellent properties, some of our compounds registered activity against mosquitoes.

These tests were made with cotton cloth stockings treated (at the rate of 3 g./ft.^{2}) with a solution of the compound. After the solvent had evaporated, the stockings were tested on a human arm in a cage of Aedes aegypti according to the following method.[47] "Small cylindrical cages 1-3/4 inches in diameter and 5-1/2 inches long, were used to confine the mosquitoes on the treated surface of the stocking. Each cage contained six mosquitoes. One end of the cage was closed by a plastic slide. This end of the cage was placed in contact with the treated stocking, and the slide was opened so that the mosquitoes could land directly on the stocking to bite. The number of seconds required for 50% of the mosquitoes to bite was recorded; testing was discontinued after 60 seconds if no bites were received. An untreated stocking and a stocking treated with deet were tested concurrently. Ten cages of

mosquitoes were tested on each stocking, and the results averaged."

The results of these tests are shown in Table IV.[47]

Table IV

Time for 50% of the Mosquitoes in a Cage to Bite (RT-50) Through a Stocking Treated at 3 g./ft.^{2} (10 Cages of 6 Mosquitoes Aedes Aegypti Each). (From the Laboratory of Carroll N. Smith, Ph.D.).

Repellent	Time after treatment (minutes;average)	RT-50 (seconds)
ENT-28339-a (A003;PADSIII-77e)	50	18.8
ENT-28340-a (A013;PADSI-61h)	58	15.1
ENT-28343-a (A017;PADSI-136c)	64	15.4
Deet	36	>60[a]
Check	--	17.1
ENT-28345-Ga (A018;PADSIV-65c)	45	21.5
ENT-28342-Ga (A016;PADSIV-78h)	51	20.7
Deet	30	>60[a]
Check	--	23.0
ENT-28345 (A018;PADSI-142f)	47	30.7
ENT-28342 (A016;PADSI-139a)	53	37.4
ENT-28341 (A014;PADSI-103k)	25	51.9
Deet	43	60[a]
Check	--	27.6

[a]No bites in 60 seconds.

Compared with the RT-50 (average time for 50% of the mosquitoes to bite) on check (untreated) stockings (i.e., 17.1-

27.6 seconds, determined for three different series of tests) and that for deet (N,N-diethyl-m-toluamide; RT-50 > 60 seconds), compound A014 (ENT-28341) (RT-50, 51.9 seconds) and compound A016 (ENT-28342) (RT-50, 37.4 seconds) were most effective among our derivatives. The RT-50 values for all other compounds tested fell in the same range as the check-determinations.

While we are most interested in developing agents surpassing in effectiveness those currently in use, immediately, we are concerned with the relative potency of our synthetic entities with respect to each other; the importance of this approach in finding significant leads to the solution of the more practical aspects of the problem cannot be overestimated. We are therefore not particularly concerned, at this time, with the fact that none among our first six compounds[1] yielded favorable results in U.S.D.A.'s standard mosquito-repellent screening test for practical application.[47]

The insect-repellent properties of our compounds are also being studied by Mr. Philip Kashin (Associate Biochemist, IIT Research Institute). He employs electronic monitoring of the biting activity of Aedes aegypti mosquitoes using mice treated with the test compounds.[48-52] While the evaluations from Mr. Kashin's laboratory indicate measurable insect-repellency for

compounds A013, A014 and A016, additional experimental work will be required -some of which is in progress- prior to making any conclusive statements.[53]

PART SIX

SUMMARY OF CURRENTLY AVAILABLE DATA ON THE ANTIDERMATOPHYTIC PROPERTIES OF OUR COMPOUNDS

In the preliminary evaluation of the antidermatophytic activity of our compounds, their inhibitory effects upon the growth of pellet cultures of Trichophyton mentagrophytes and of slant cultures of Trichophyton mentagrophytes, Trichophyton rubrum and Keratinomyces ajelloi were determined. The work is being carried out in the laboratories of Doctor Robert G. Crounse (Chairman, Division of Dermatology, Johns Hopkins University School of Medicine).

In these tests the evaluant was incorporated in the test media at concentrations of 5 μg./ml., 10 μg./ml. and 20 μg./ml. Corresponding control determinations were carried out simultaneously. In each case, the colony diameter was determined at periodic intervals and a plot made of this parameter vs. the time (in days) when the measurement was made. The results of these preliminary tests are shown in Figures 12-15.

While varying degrees of inhibition were elicited by the compounds tested, the inhibitory effect of compound A013 (20 μg./ml.) on the slant culture of Trichophyton mentagrophytes (Figure 12), that of compounds A003 (10 μg./ml.) and

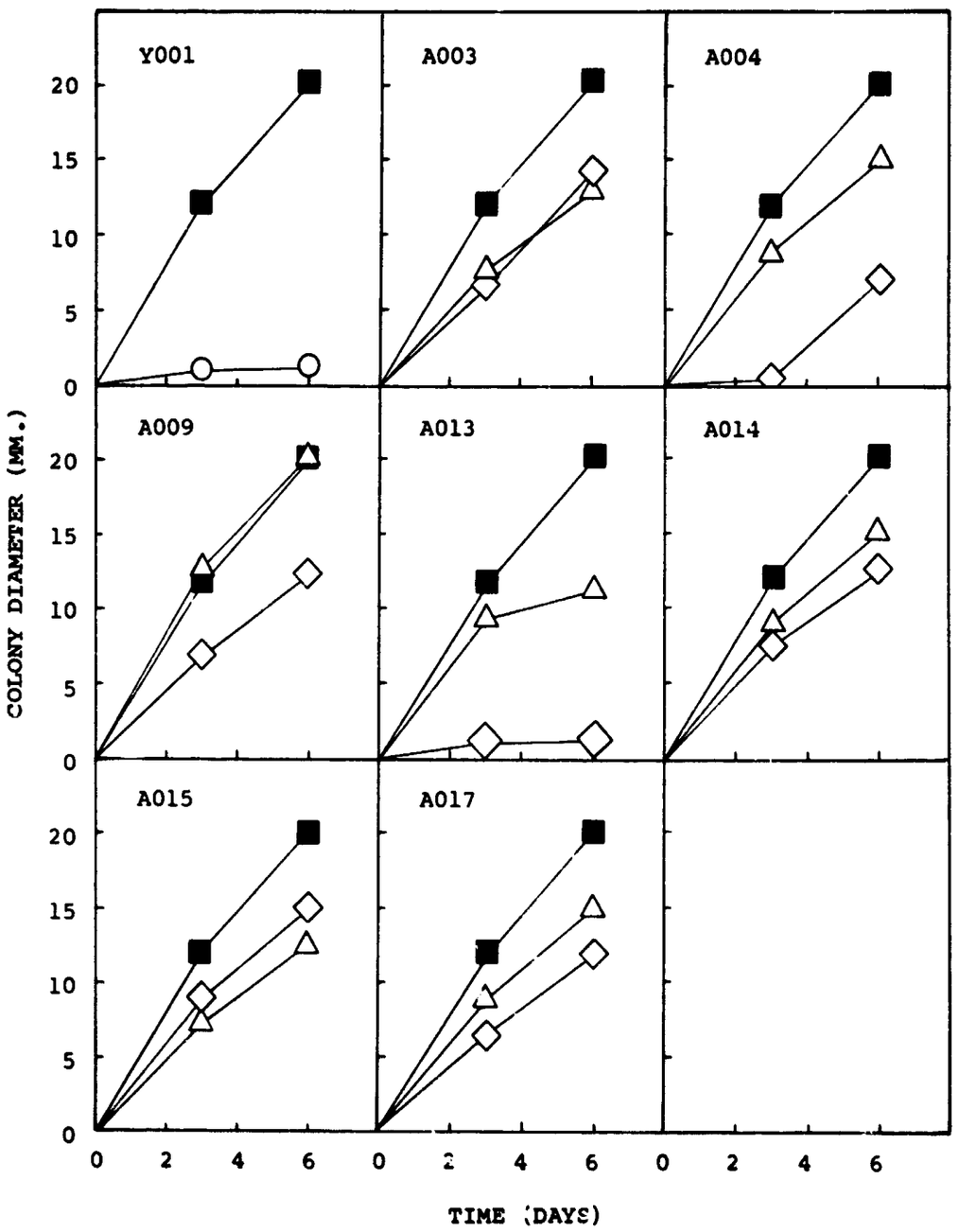

Fig. 12.— Inhibition of Trichophyton mentagrophytes (slant culture) by our compounds at concentrations: ○, 5 µg./ml.; △, 10 µg./ml.; ◇, 20 µg./ml. The control, ■, was obtained by averaging the results of three experiments. (From the laboratory of Robert G. Crounse, M.D.).

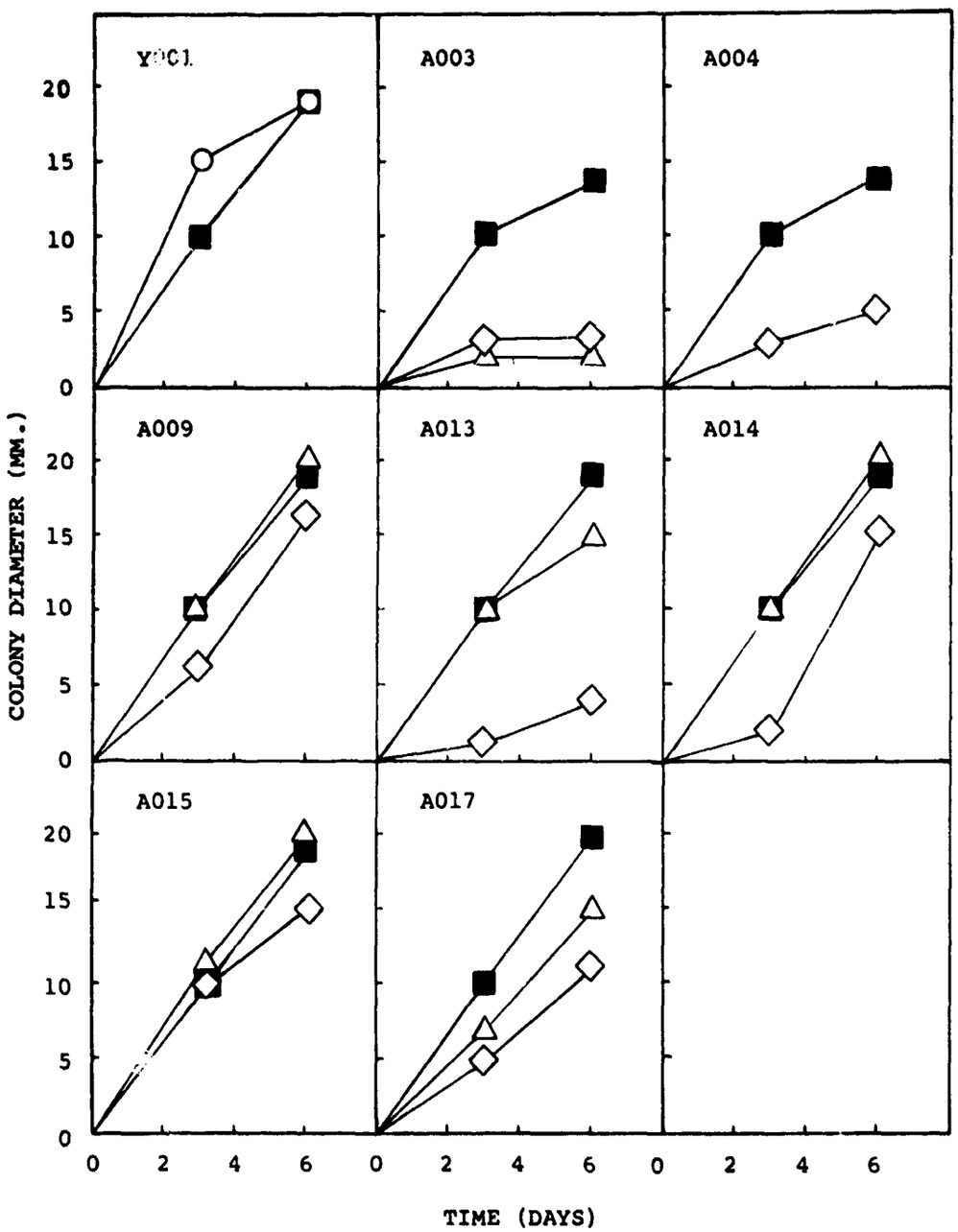

Fig. 13.— Inhibition of Keratinomyces ajelloi (slant culture) by our compounds at concentrations: ○, 5 µg./ml.; △, 10 µg./ml.; ◇, 20 µg./ml. The control, ■, was obtained by averaging the results of three experiments. (From the laboratory of Robert G. Crounse, M.D.).

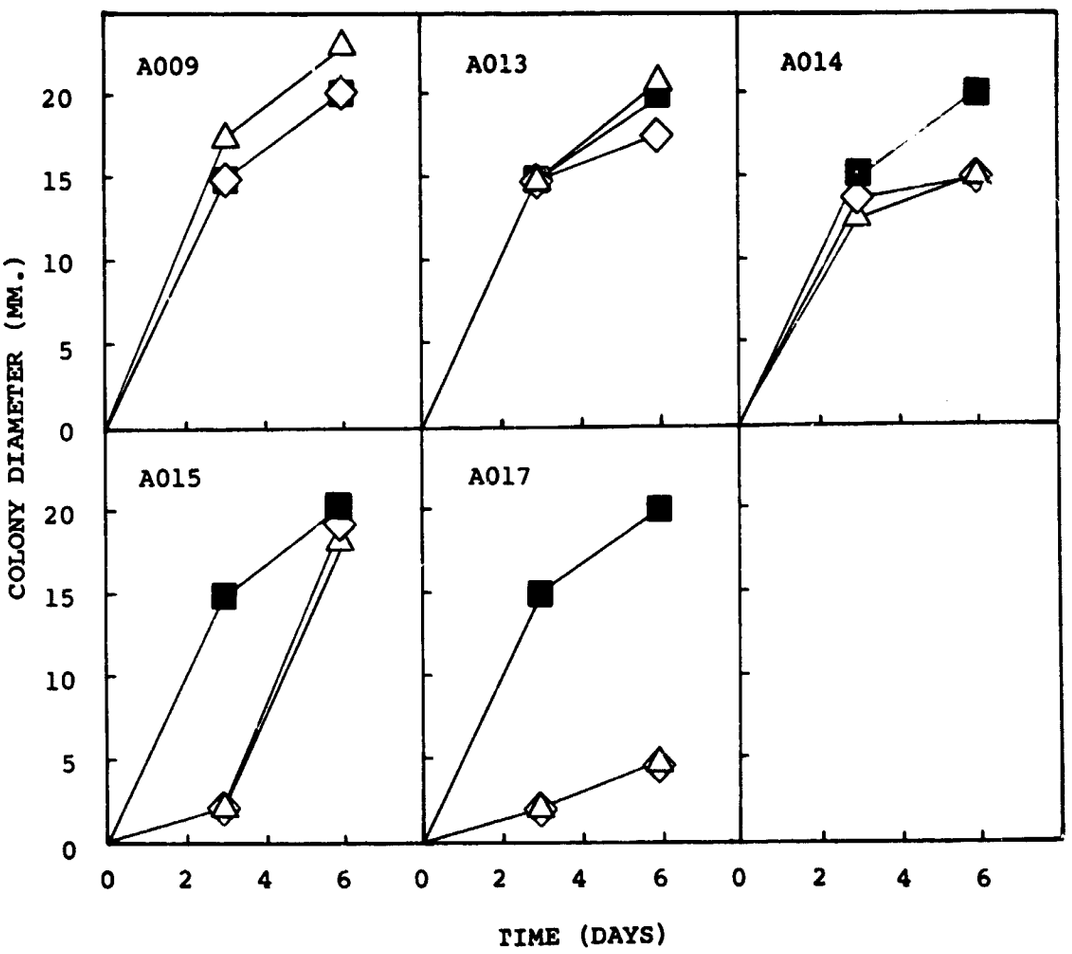

Fig. 14.— Inhibition of Trichophyton rubrum (slant culture) by our compounds at concentrations: △, 10 µg./ml.; ◇, 20 µg./ml. The control, ■ was obtained from results of a single experiment. (From the laboratory of Robert G. Crounse, M.D.).

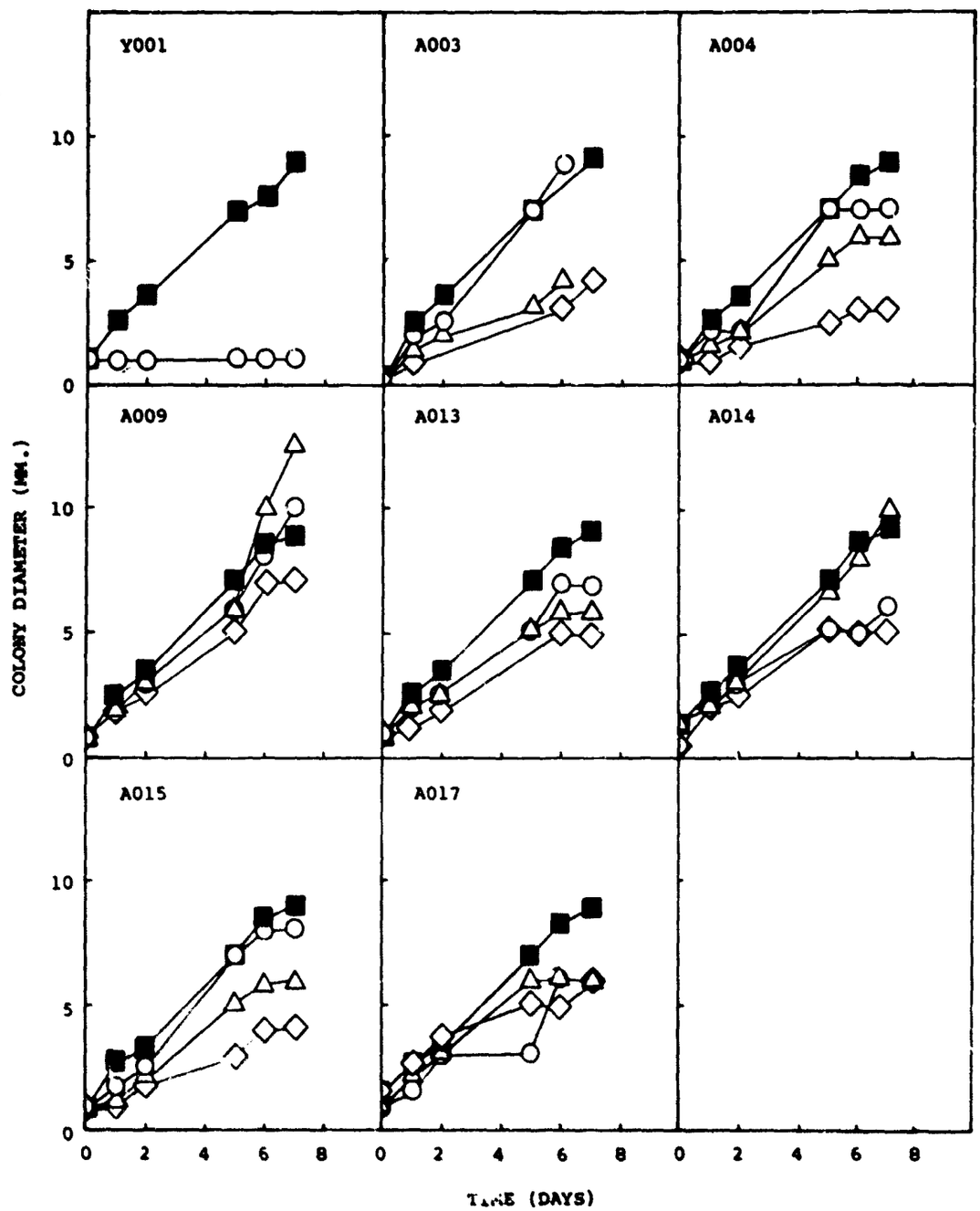

Fig. 15.— Inhibition of Trichophyton mentagrophytes (pellet culture) by our compounds at concentrations: ○, 5 µg./ml.: △, 10 µg./ml.: ◇, 20 µg./ml. The control, ■, was obtained by averaging the results experiments. (From the laboratory of Robert G. Crounse, M.D.).

A013 (20 μg./ml.) on the slant culture of Keratinomyces ajelloi (Figure 13) and that of compound A017 (20 μg./ml.) on the slant culture of Trichophyton rubrum (Figure 14) are particularly noteworthy.

The relative influence our compound A003 has upon a slant culture of Trichophyton mentagrophytes is effectively depicted in Figure 16.

Even though higher concentrations of our compounds may be necessary to effect significant growth inhibition in these tests, it is possible that enhanced localization of these substances in skin tissue could render them therapeutically more effective than, for example, griseofulvin itself. Furthermore, there is a distinct possibility that some of our compounds' spectral range of antidermatophytic activity may differ significantly from that of the currently available chemotherapeutic agents.

We find, too, the poor inhibitory action of compound A009 interesting; the removal of the third ring component in griseofulvin is associated with a dramatic reduction of in vitro antidermatophytic activity.

It should be pointed out that the work reported herein is

being expanded to include other dermatophyte test organisms as well as additional techniques.

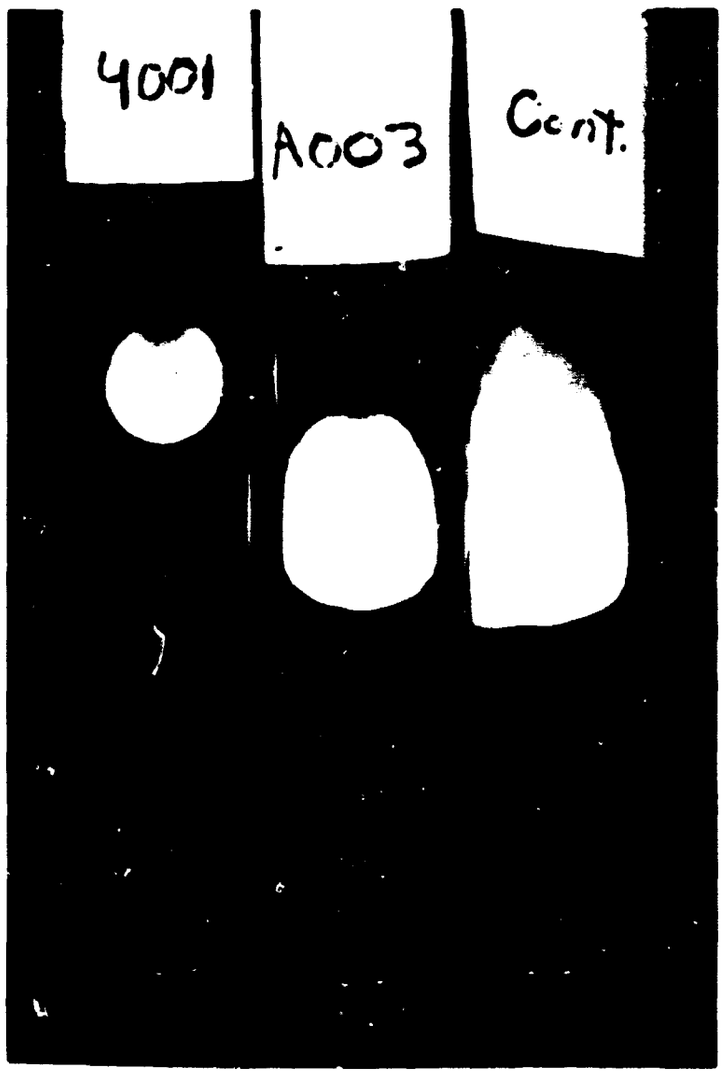

Fig. 16.— Illustration of inhibitory effect of our compound A003 and compound Y001 (griseofulvin) upon Trichophyton mentagrophytes (slant culture). The tube at the right shows the growth of the control colony. The age of the cultures is the same in each case. (From the laboratory of Robert G. Crounse, M.D.).

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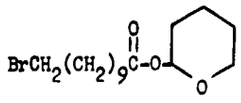
APPENDIX

Formula Review, Infrared and Ultraviolet Absorption Spectra, Pressure-Area Curves from Monolayer Studies and Reproduction of Published Communication.



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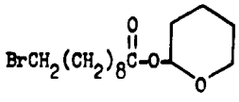

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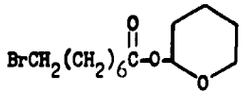

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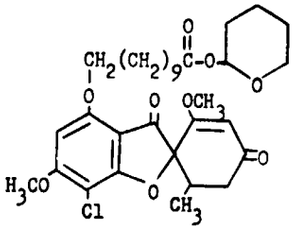

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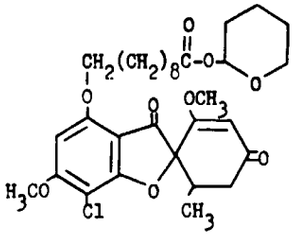

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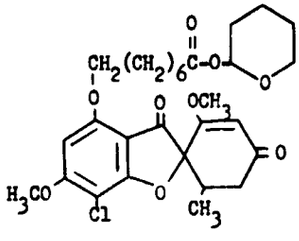

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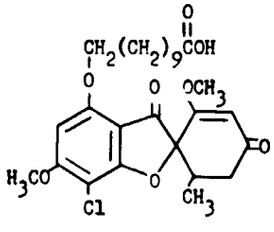

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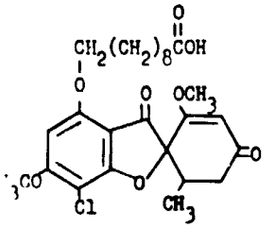

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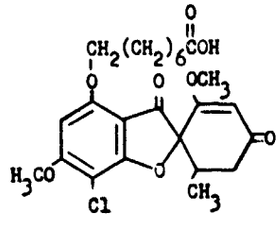

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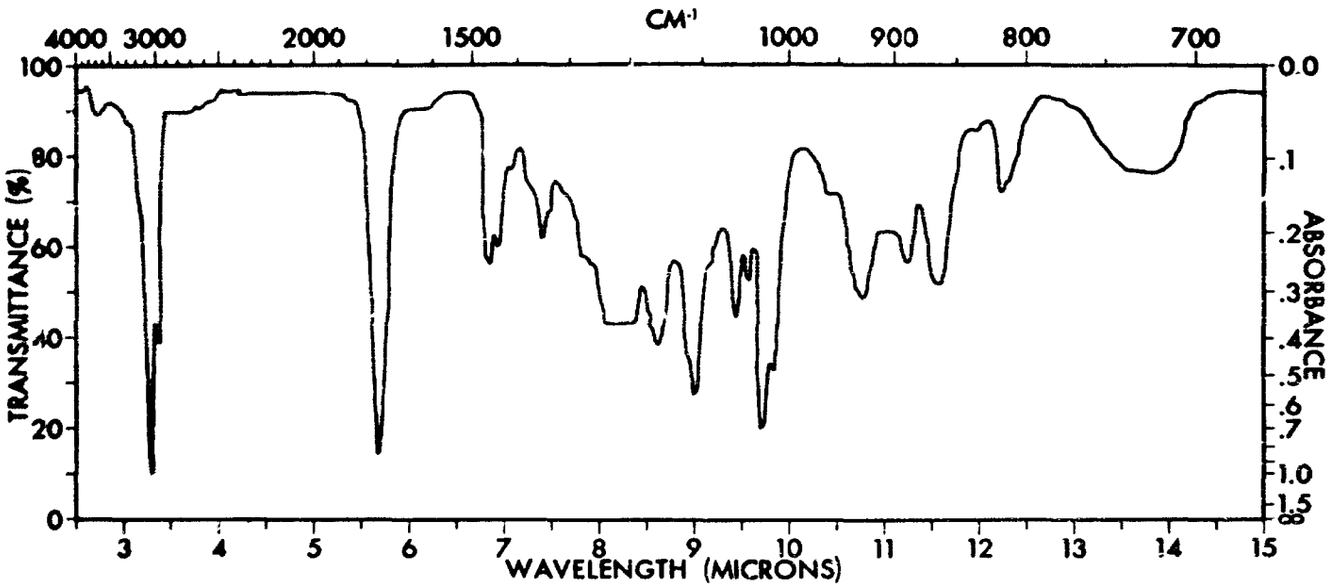
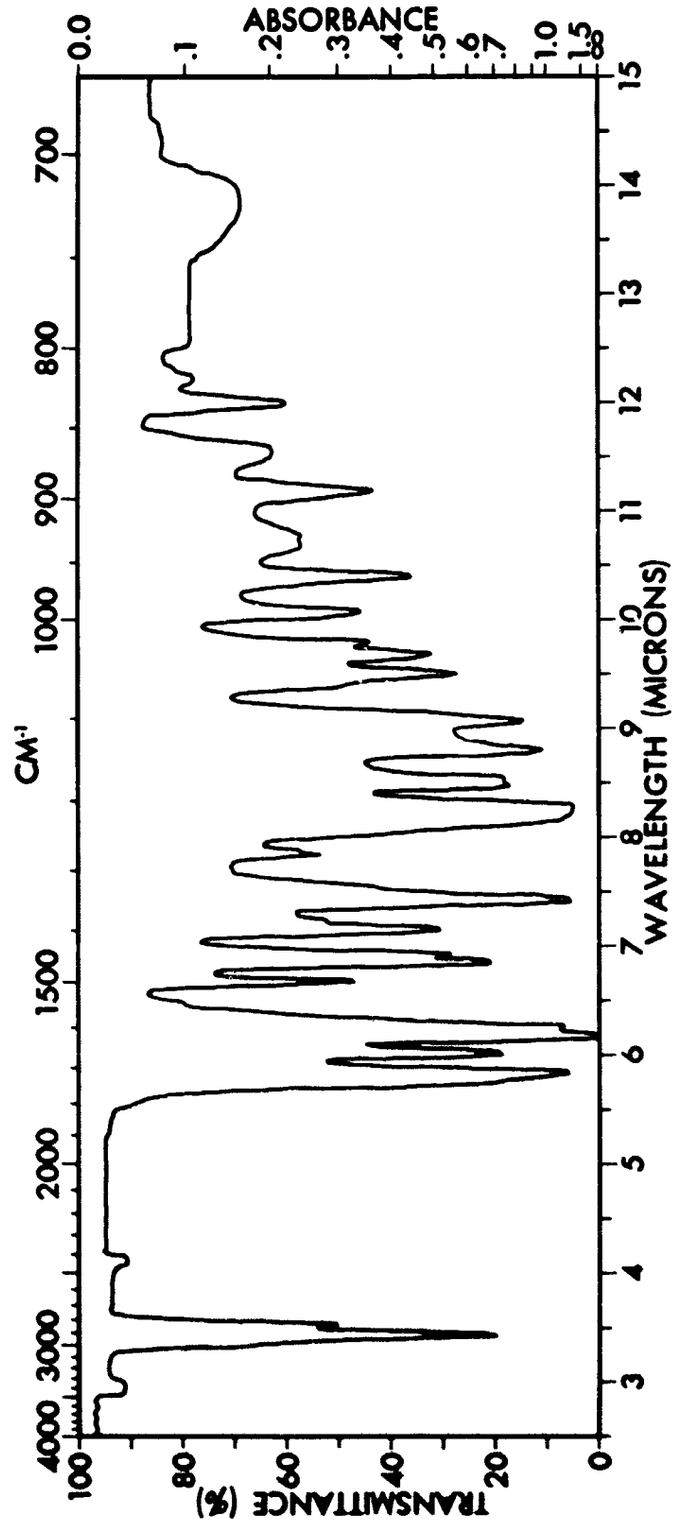
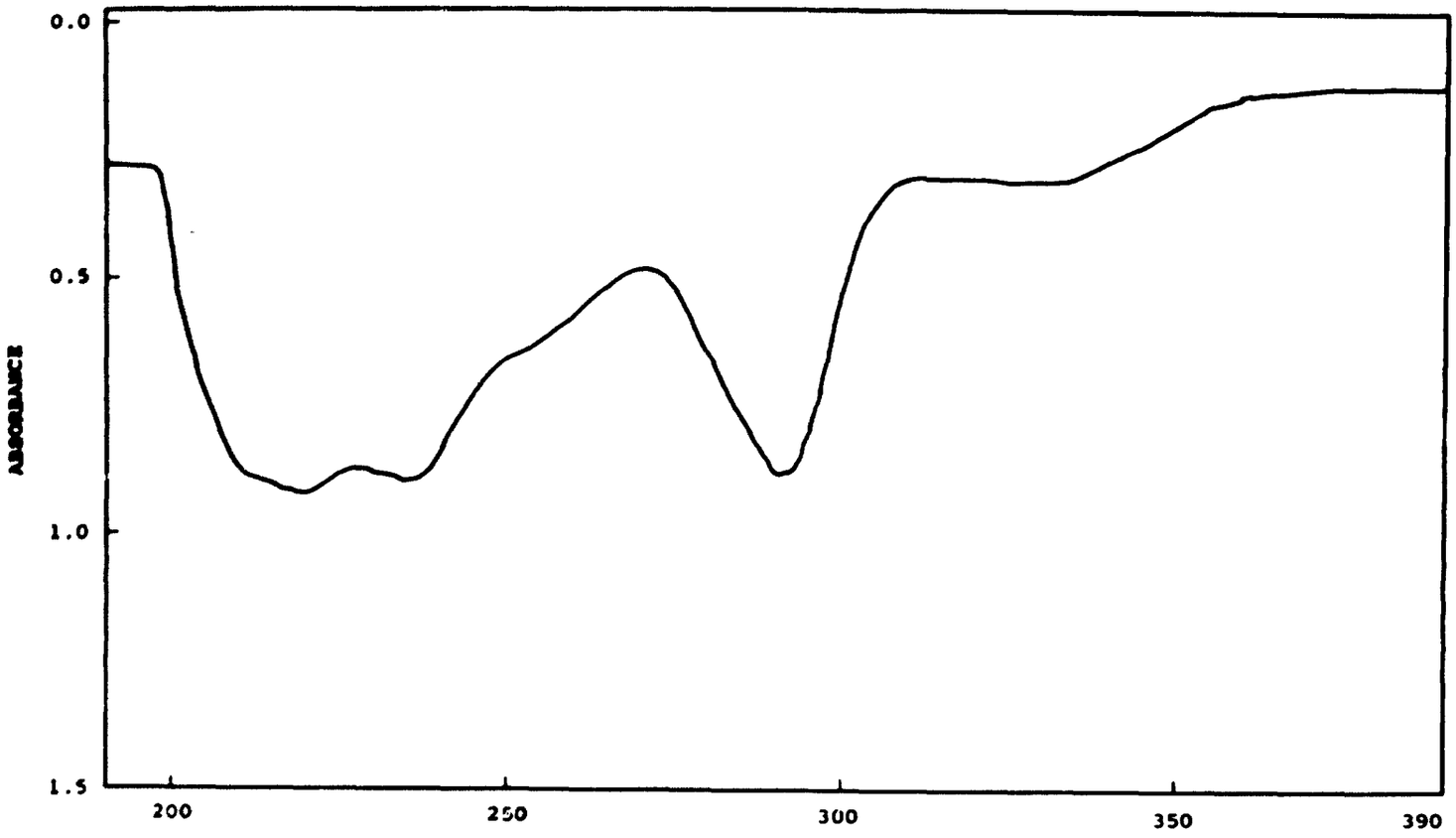
Formula Review to Part Two.



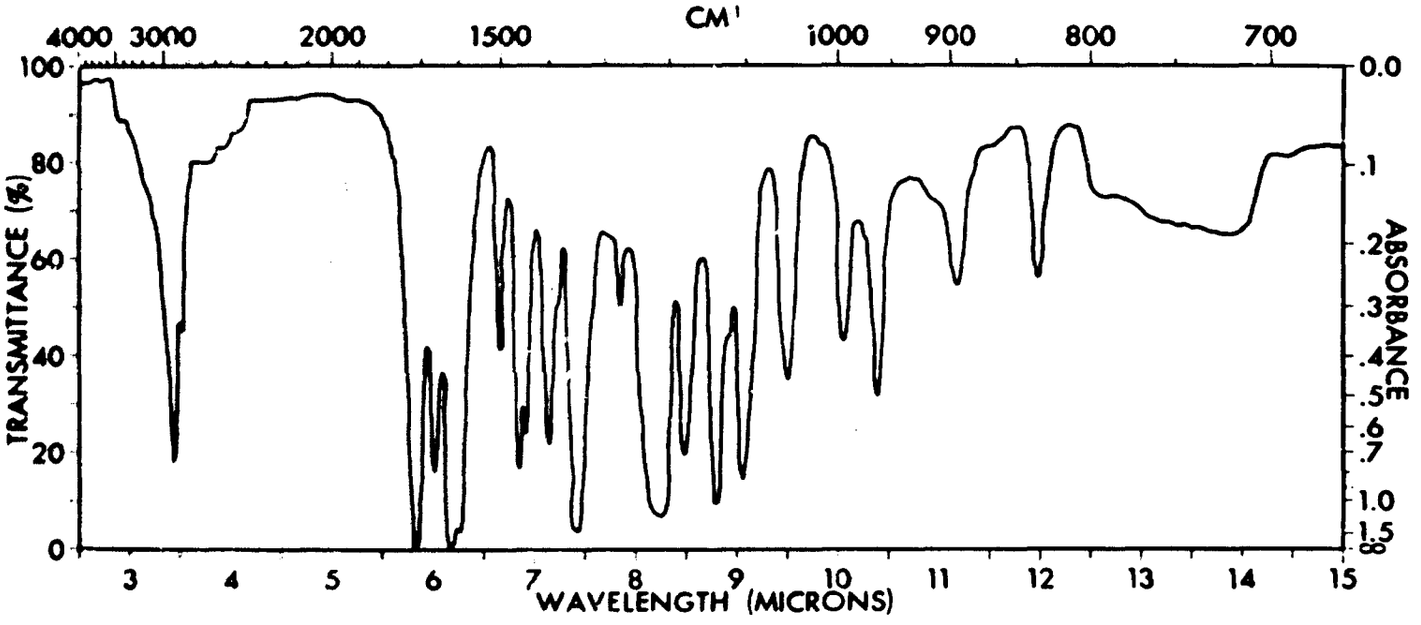
AO27;PADSIII-137c
Phase: Chloroform Solution
Thickness: 0.10 mm.



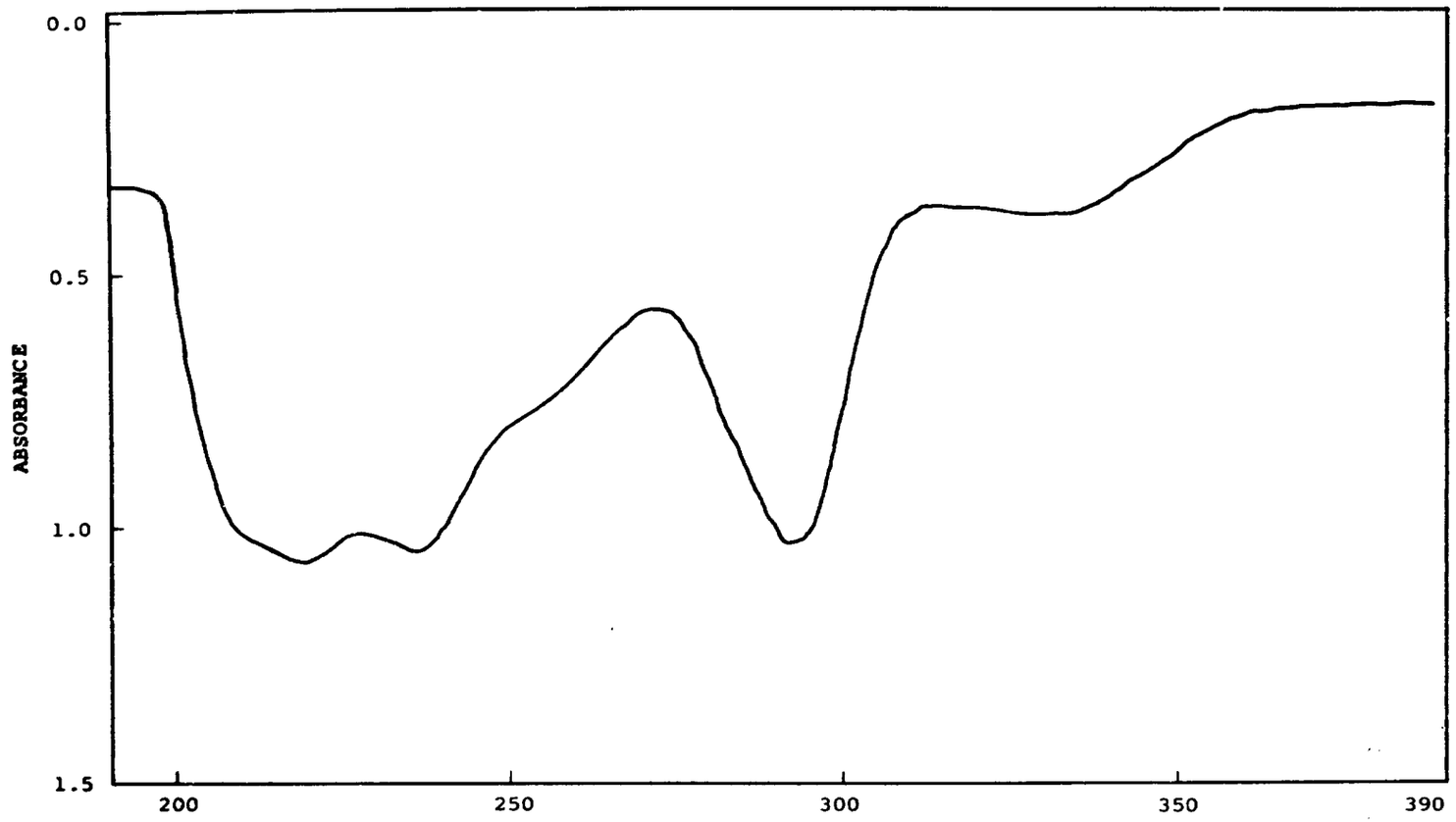
AO28;PADSV-2k
Phase: Chloroform Solution
Thickness: 0.10 mm.



AO28;PADSV-2k
Solvent: 95% Ethanol
Concentration: 3.29×10^{-5} M
Cell Path: 1 cm.



AO29;PADSIII-151i
Phase: Chloroform Solution
Thickness: 0.10 mm.



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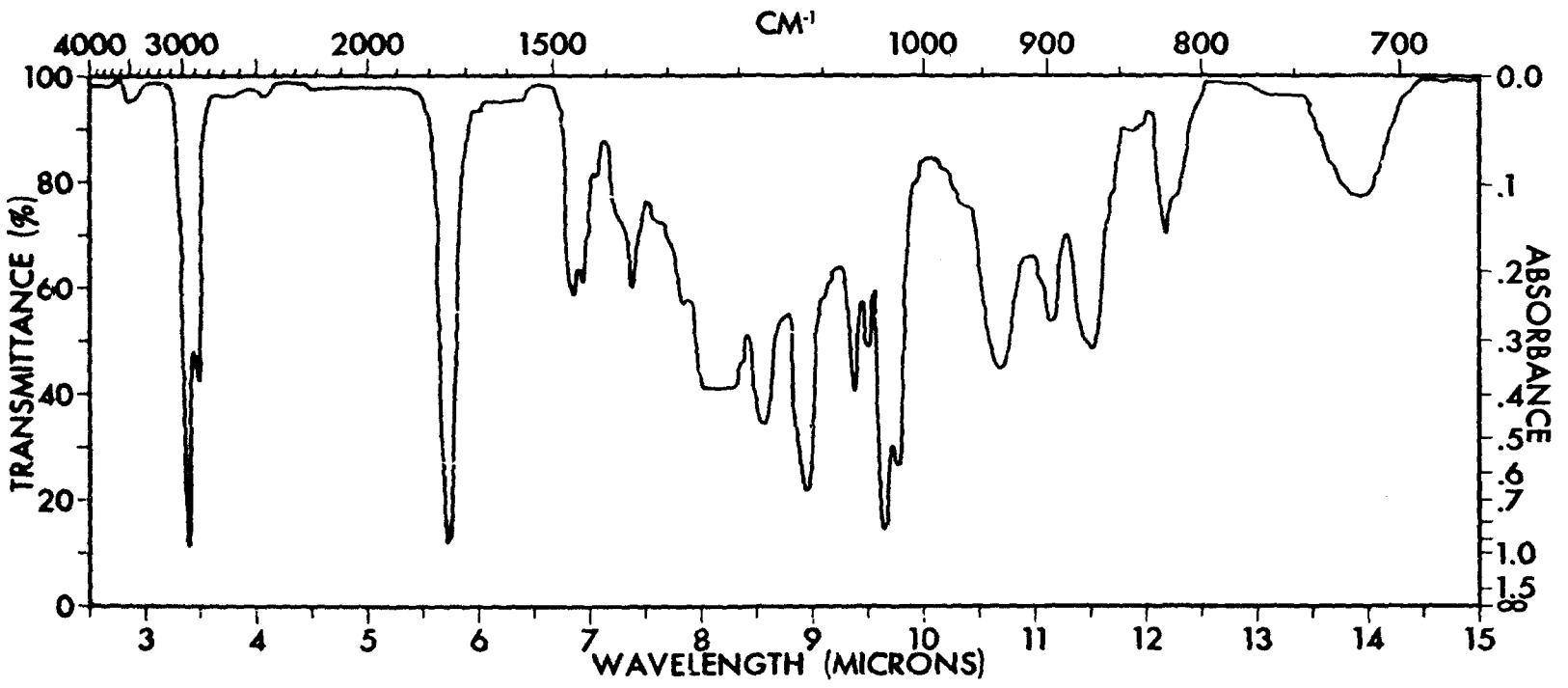
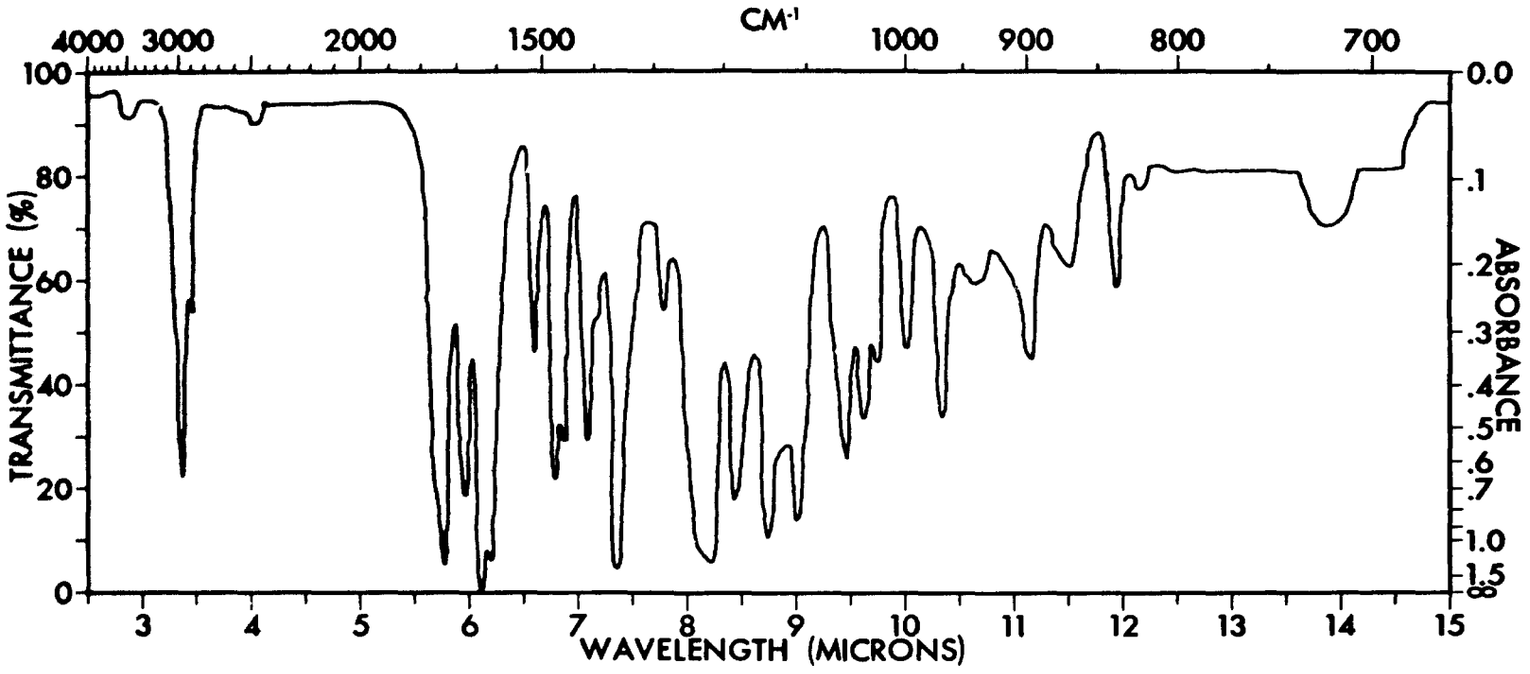
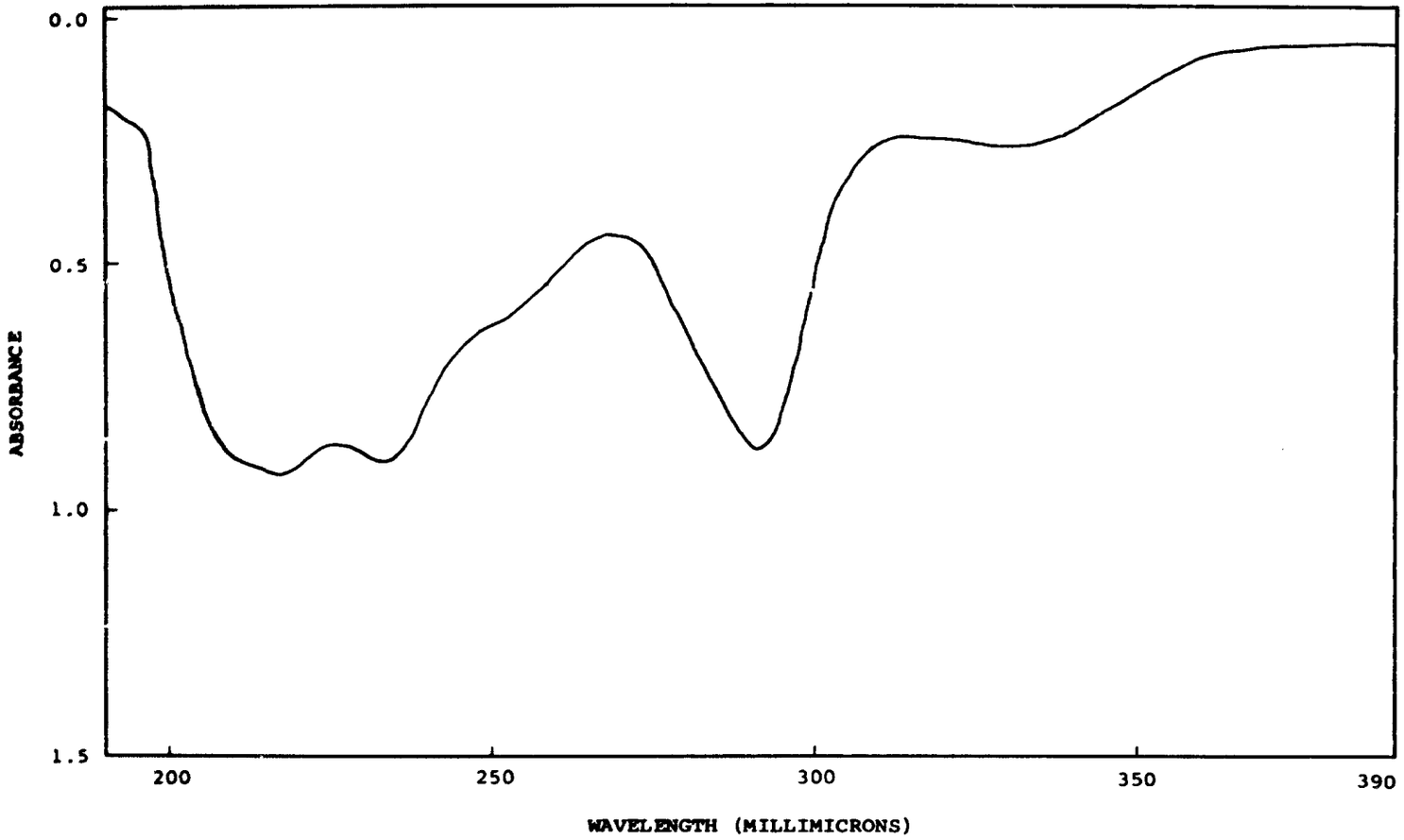
Solvent: 95% Ethanol
Concentration: 3.82×10^{-5} M
Cell Path: 1 cm.



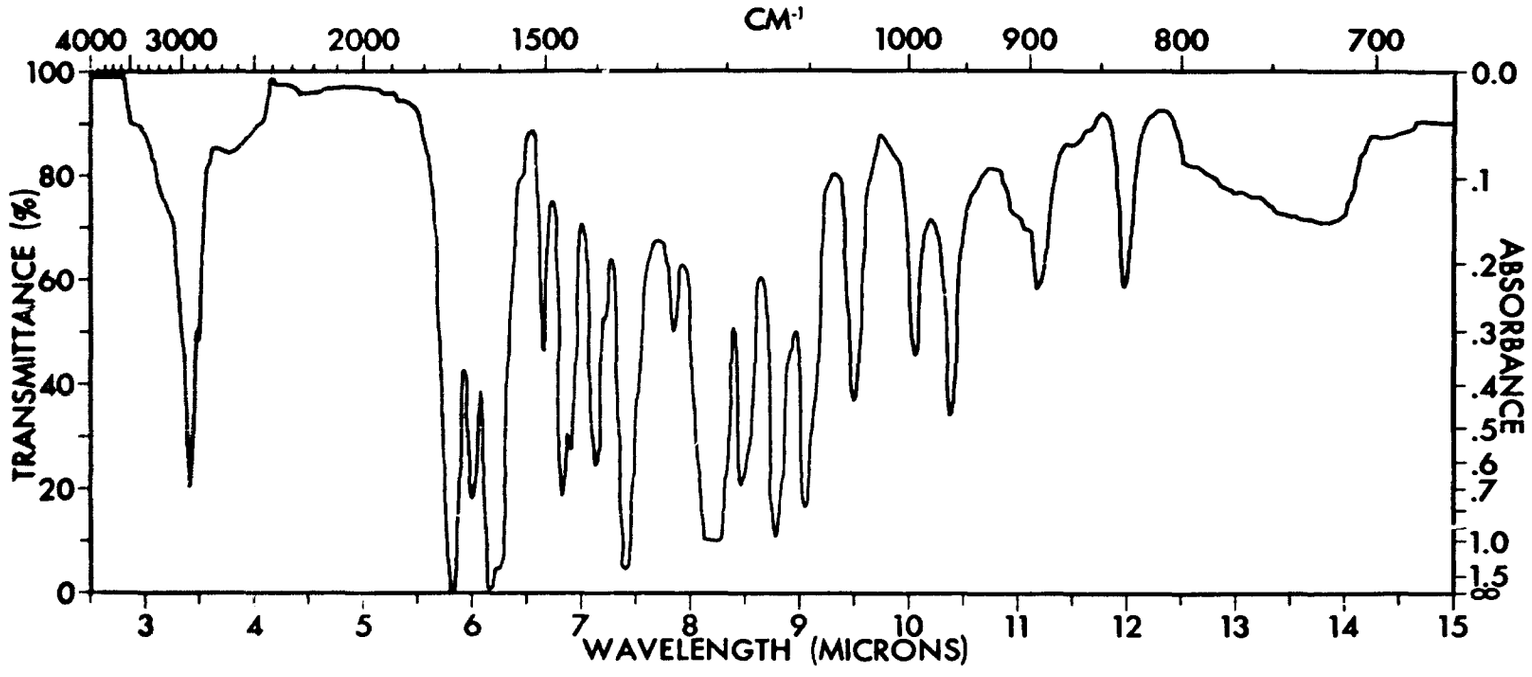
AO31;PADSIV-111e
Phase: Chloroform Solution
Thickness: 0.10 mm.



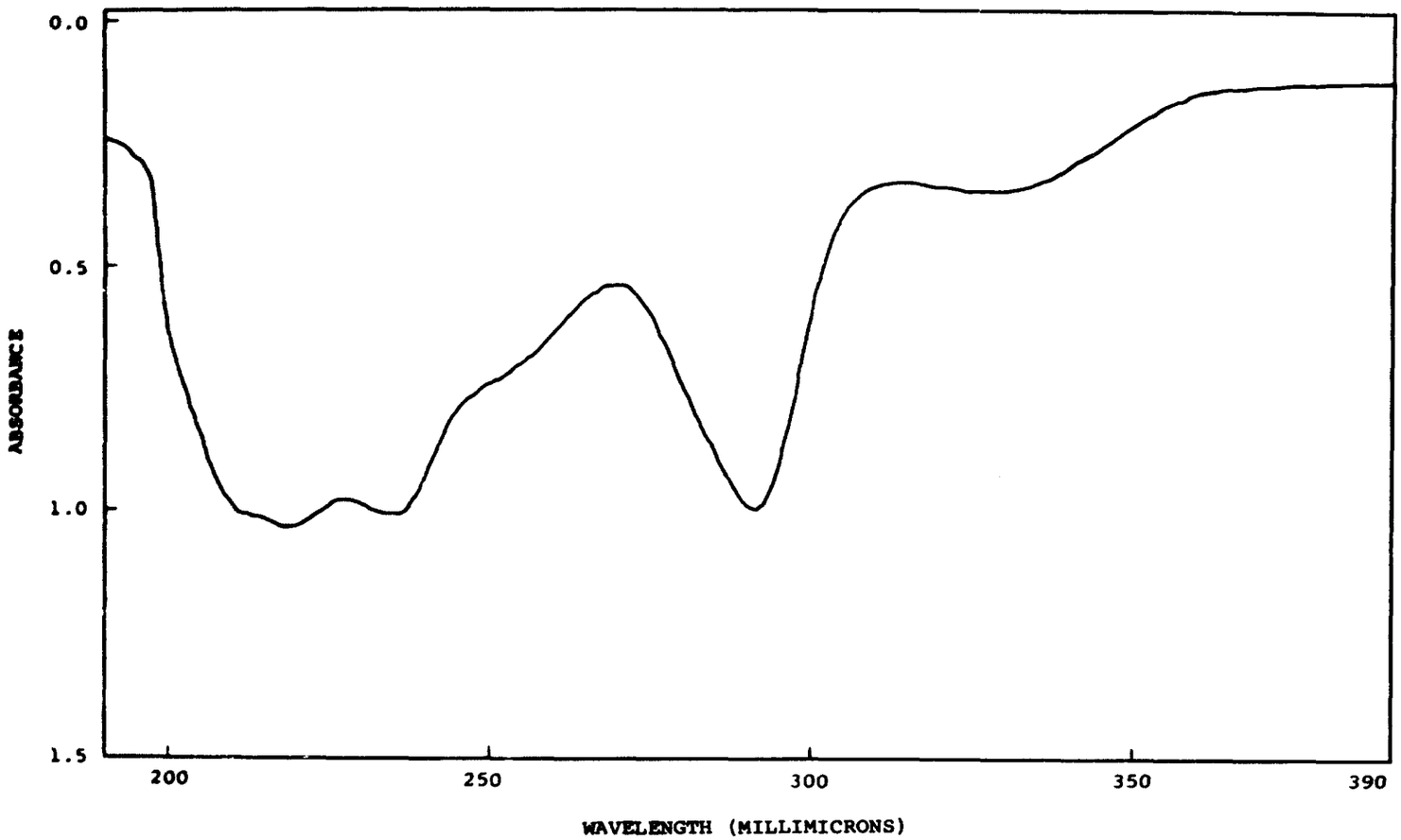
AO32;PADSIV-121e
Phase: Chloroform Solution
Thickness: 0.10 mm.



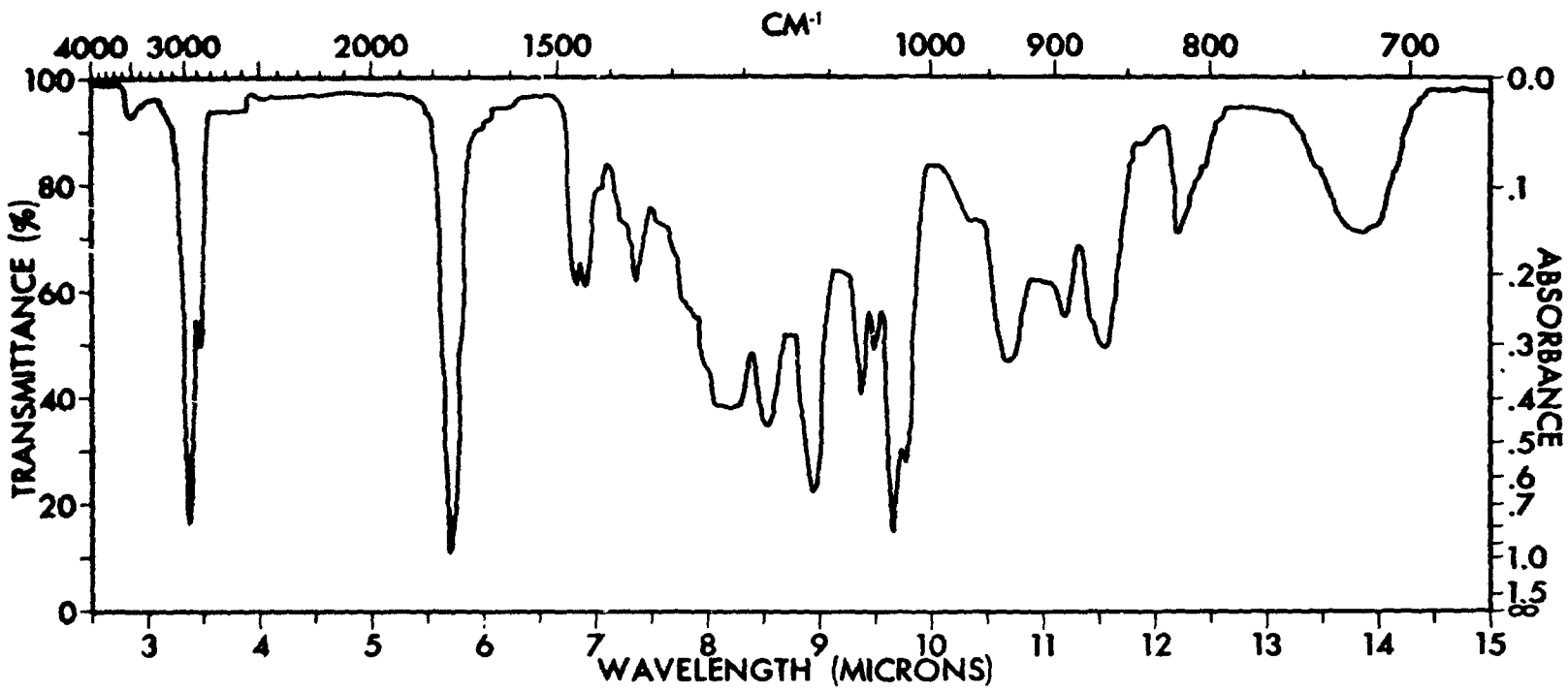
AO32;PADSIV-121e
Solvent: 95% Ethanol
Concentration: 3.37×10^{-5} M
Cell Path: 1 cm.



AO33;PADSV-38f
Phase: Chloroform Solution
Thickness: 0.10 mm.

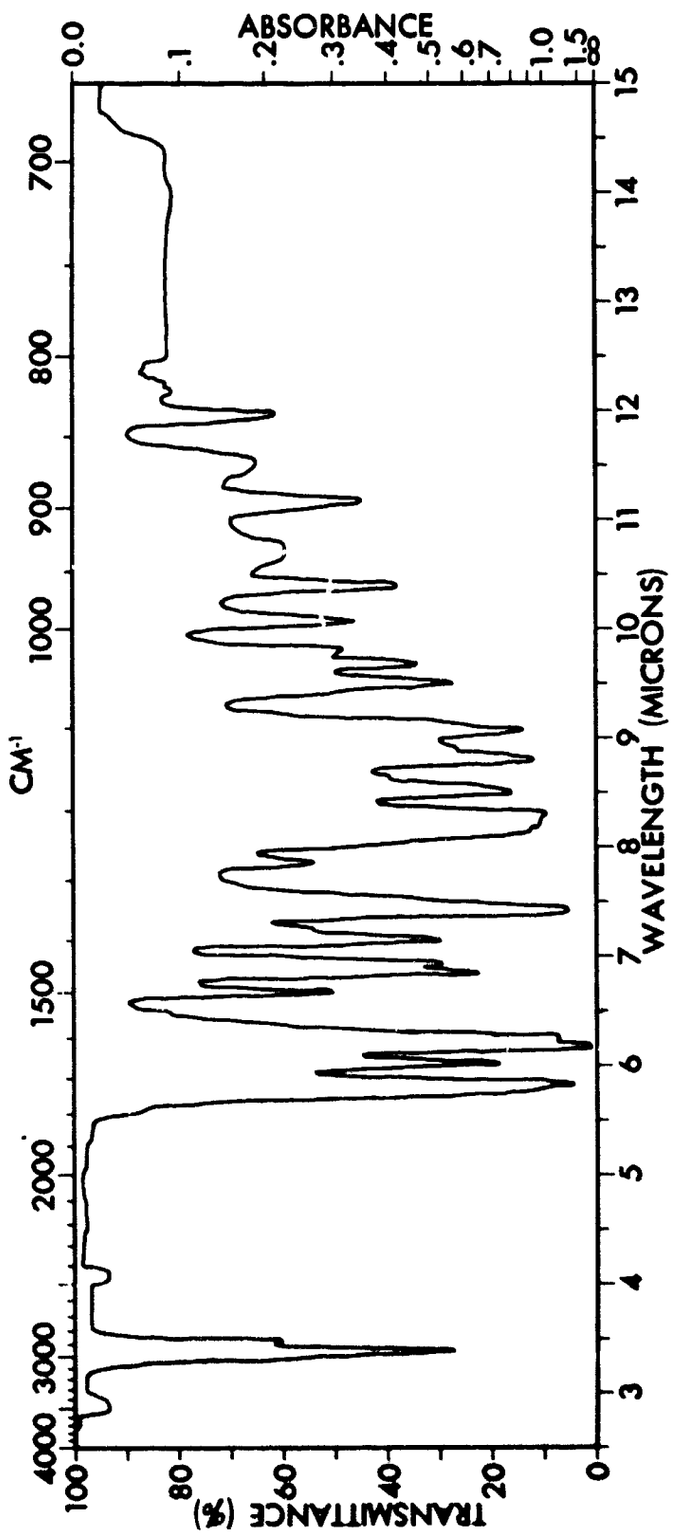


AO33;PADSV-38f
Solvent: 95% Ethanol
Concentration: 3.93×10^{-5} M
Cell Path: 1 cm.

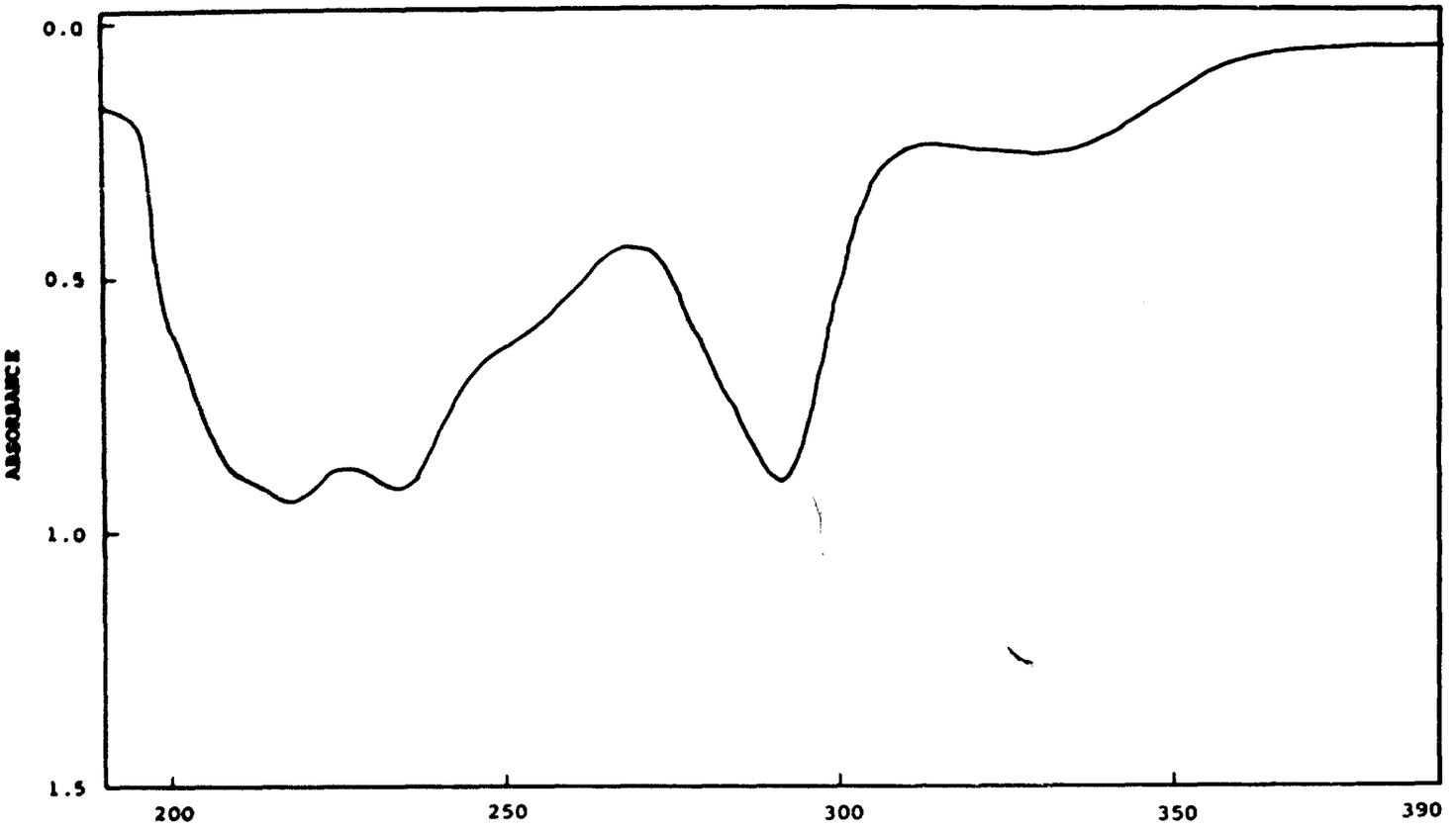


A034;PADSIV-71

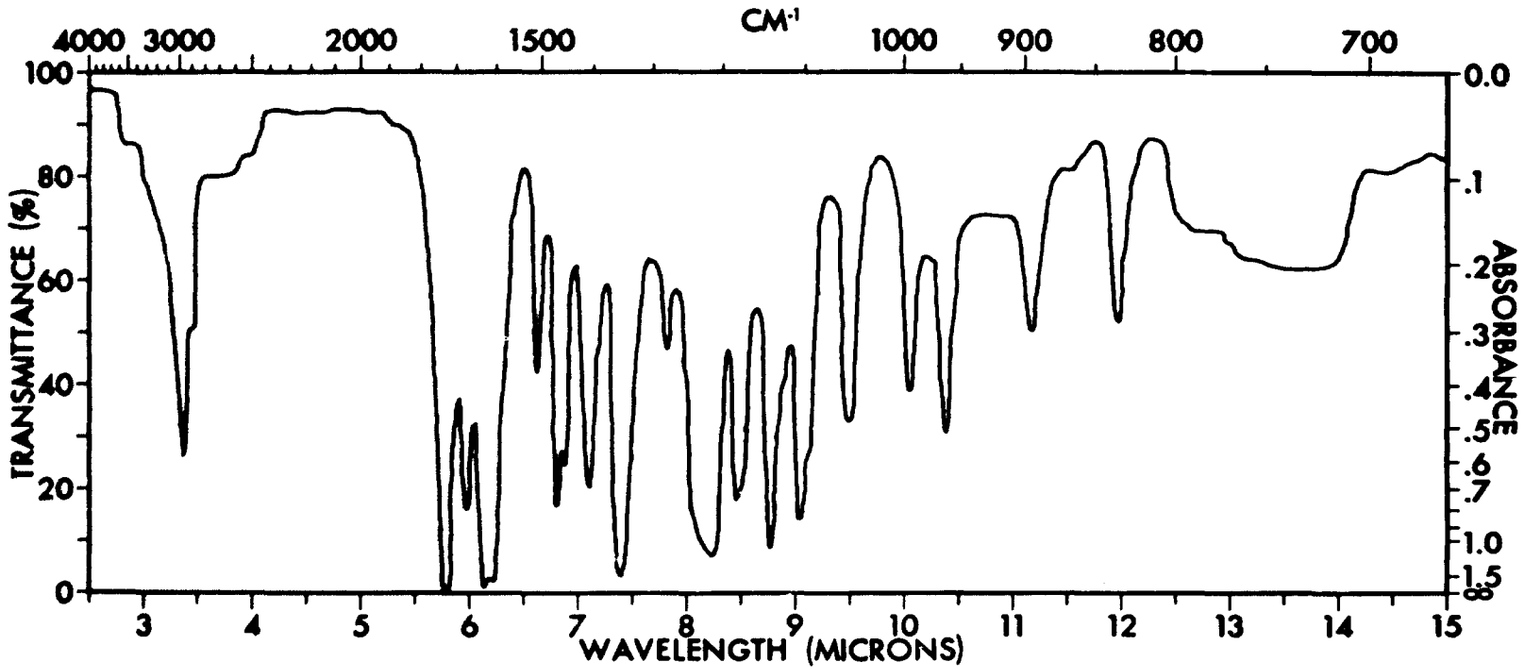
Phase: Chloroform Solution
Thickness: 0.10 mm.



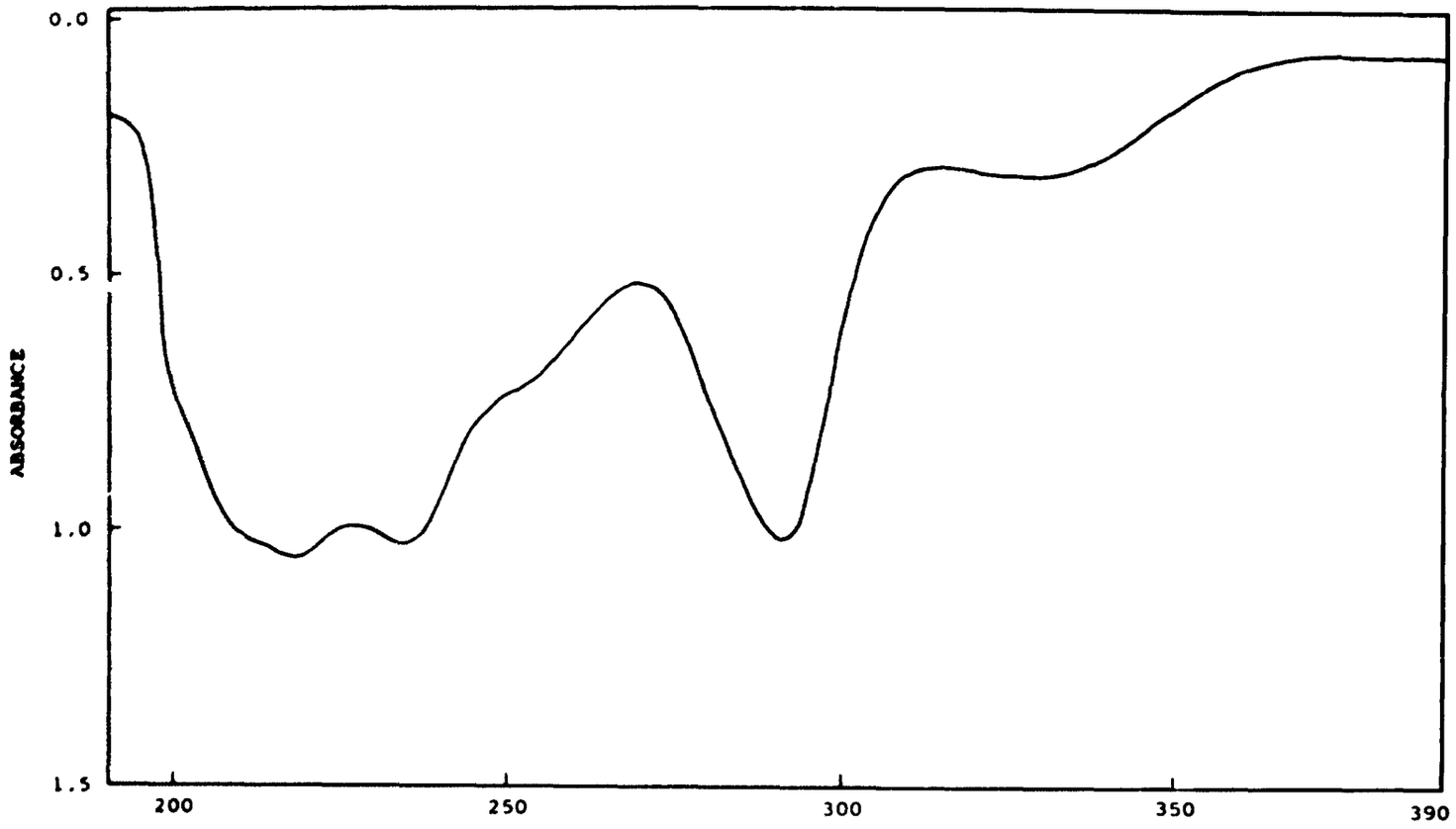
AO35;PADSIV-101b
Phase: Chloroform Solution
Thickness: 0.10 mm.



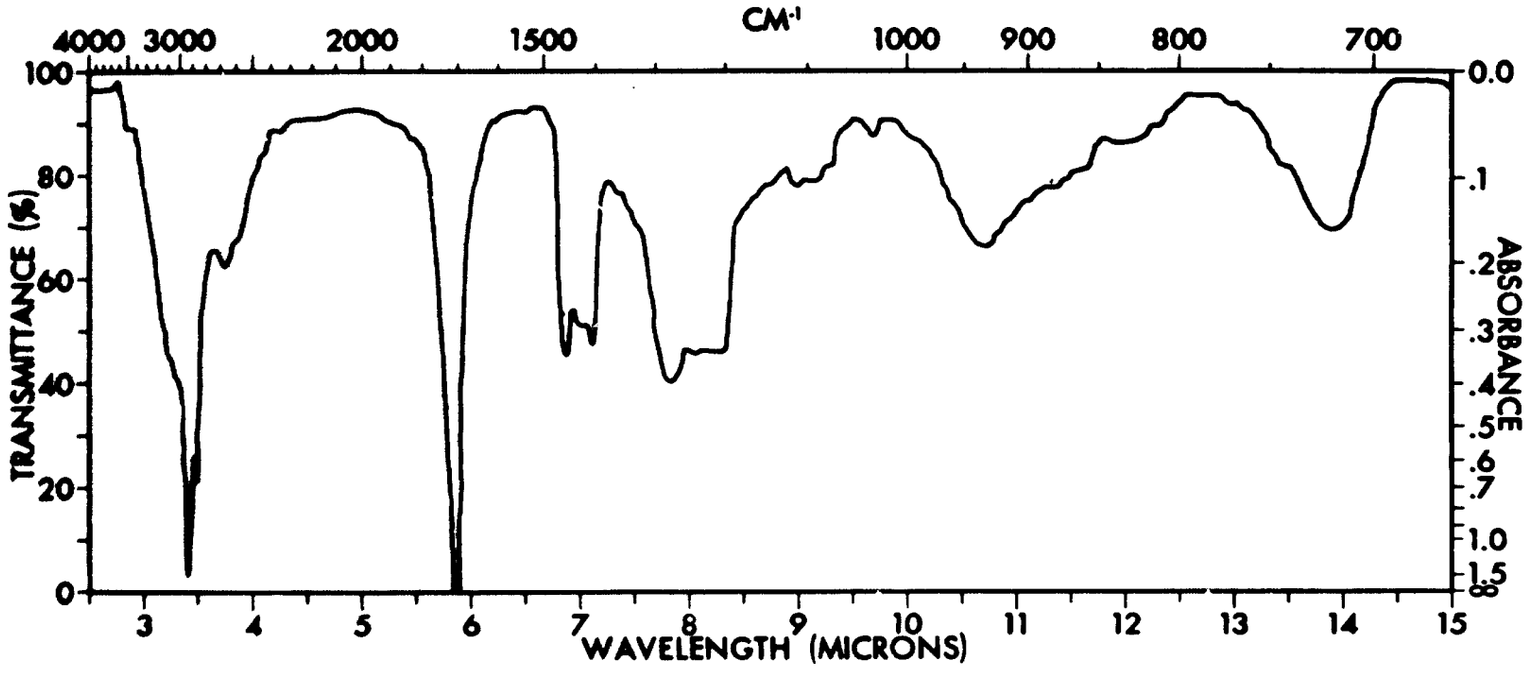
AO35;PADSIV-124j
Solvent: 95% Ethanol
Concentration: 3.54×10^{-5} M
Cell Path: 1 cm.



AO36;PADSIV-131e
Phase: Chloroform Solution
Thickness: 0.10 mm.



AO36;PADSIV-131e
Solvent: 95% Ethanol
Concentration: 4.16×10^{-5} M
Cell Path: 1 cm.



YOO3;PADSIII-44b
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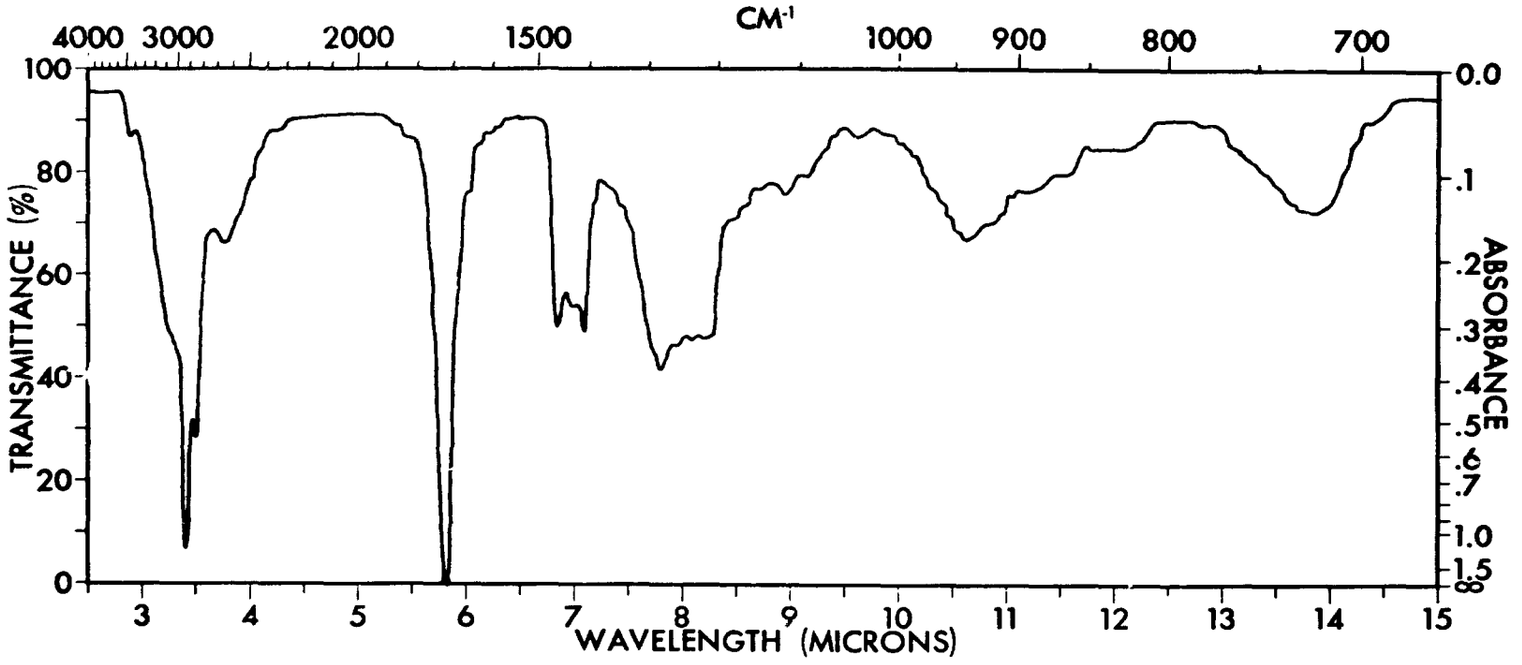

Y004;PADSV-19b
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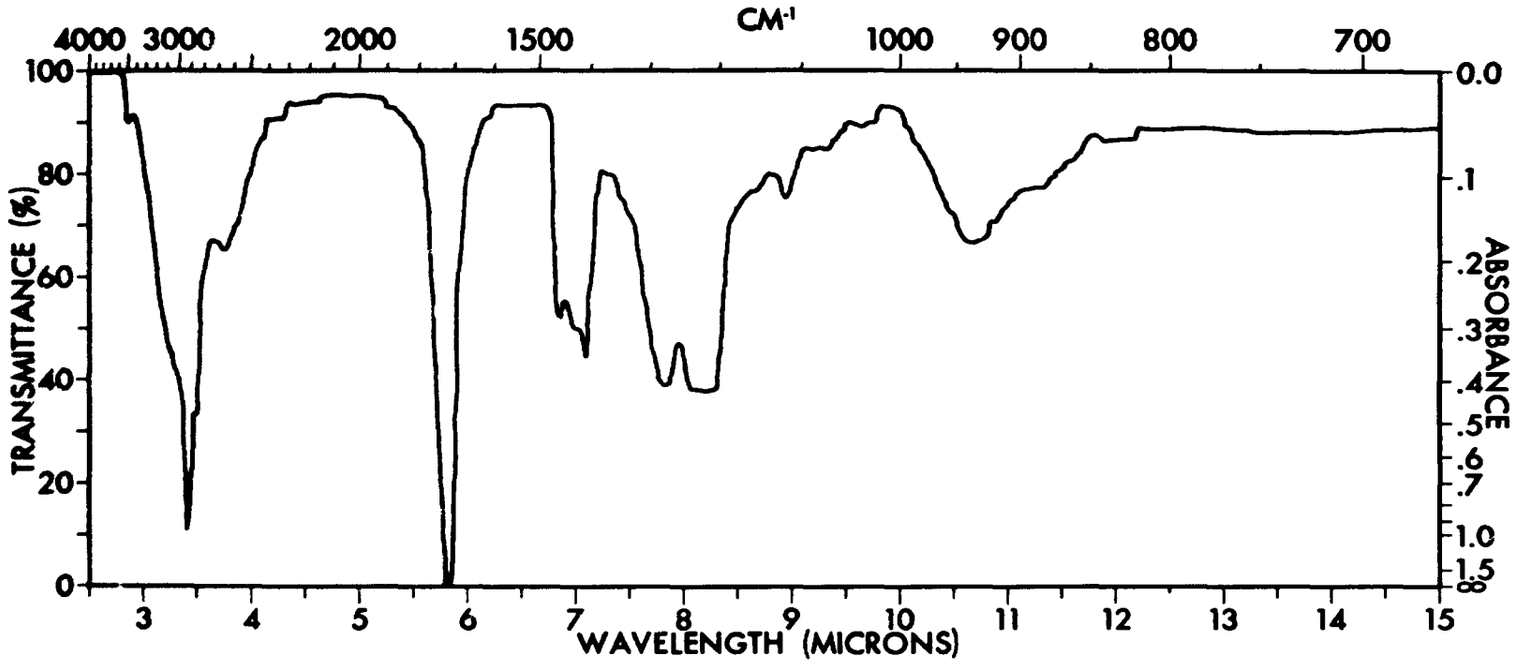
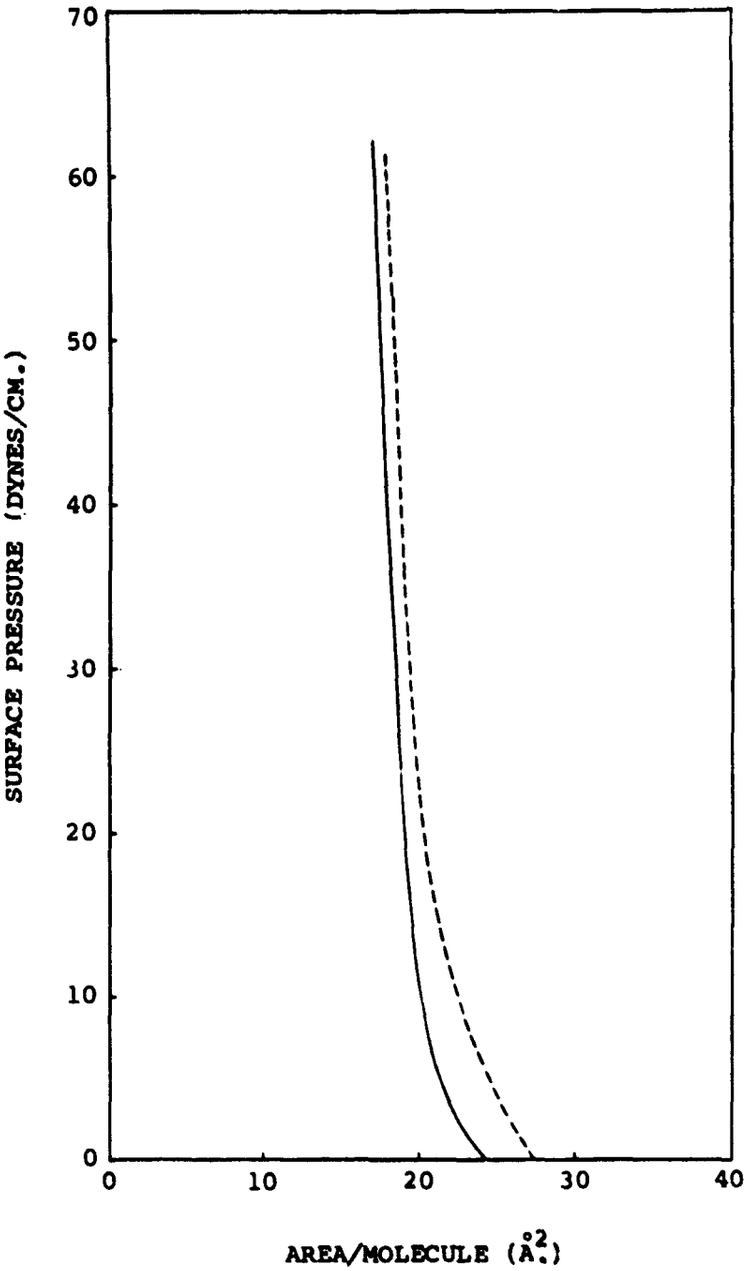
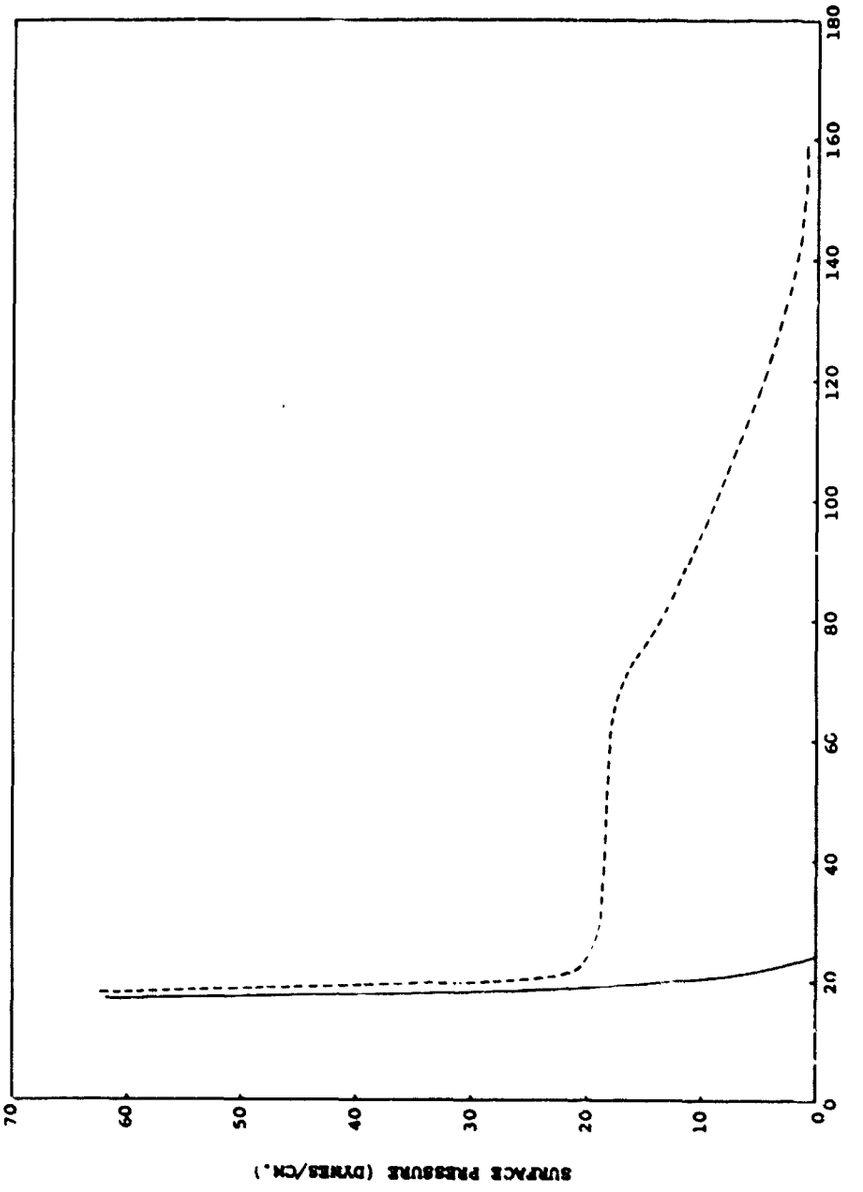


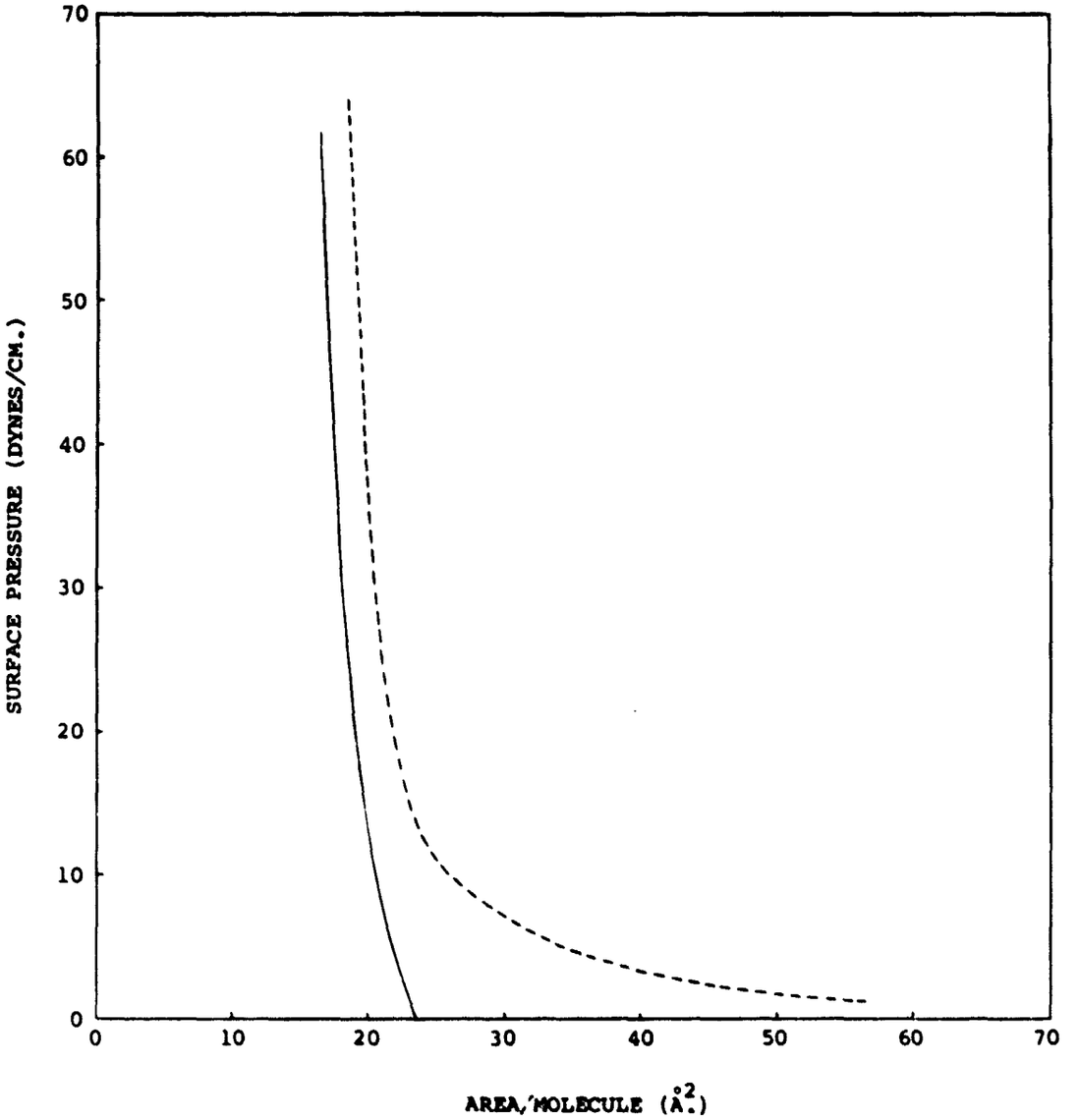
Y005;PADSIV-73b
Phase: Chloroform Solution
Thickness: 0.10 mm.



Pressure-area curves for stearic acid (——) and an equimolar mixture of stearic acid and compound A013 (---).



Pressure-area curves for stearic acid (——)
and an equimolar mixture of stearic acid
and compound A014 (---).



Pressure-area curves for stearic acid (——) and an equimolar mixture of stearic acid and compound A029 (---).

[Reprinted from the Journal of Medicinal Chemistry, 9, 242 (1966).]

Synthesis of Grisan and Coumaran-3-one Derivatives with Potential Insect-Repellent Properties[1]

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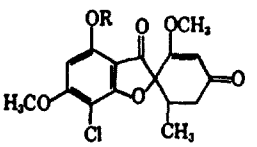
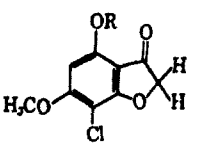
Received July 16, 1965

In developing structural designs for compounds which would prove effective as systemically administered insect repellents, we sought to combine within a *single molecule* (1) a component known to have affinity for dermal tissue with (2) a component possessing certain insect-repellent (or prophylactic or therapeutic) properties. In the initial phase of our exploratory work, we have synthesized a series of compounds (I–III and VI–VIII) combining into one molecular entity a moiety (IV) known to be transported to and found in epidermal tissue in significant quantities[2] [or a component (IX) of the latter], with moieties ascertained to have mosquito-repellent properties (anisyl alcohol,[3a] N,N-diethyl-*m*-toluamide,[3b] and citronellol[3c]).





I, R = *p*-$CH_2C_6H_4OCH_3$
II, R = *m*-$CH_3C_6H_4CON(C_2H_5)_2$
III, R = $CH_2CH_2CH(CH_3)CH_2CH_2CH{=}C(CH_3)_2$
IV, R = CH_3
V, R = H

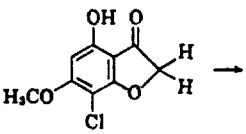
VI, R = *p*-$CH_2C_6H_4OCH_3$
VII, R = *m*-$CH_3C_6H_4CON(C_2H_5)_2$
VIII, R = $CH_2CH_2CH(CH_3)CH_2CH_2CH{=}C(CH_3)_2$
IX, R = CH_3
X, R = H

Chemistry.—The subject compounds could be viewed, basically, as condensation products of 4-demethylgriseofulvin[4] (V) and of the corresponding coumaran-3-one (X) with anisyl alcohol, N,N-diethyl-*m*-toluamide, and citronellol.

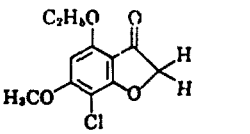
Compounds I, II, and III were actually obtained by treating V with anisyl bromide[5] (XI), 3-(N,N-diethylcarbamoyl)benzyl bromide (XII), and citronellyl bromide[6] (XIII) in acetone and/or dimethylformamide, in the presence of potassium carbonate.

7-Chloro-4-hydroxy-6-methoxycoumaran-3-one (X), a hitherto unreported moiety, was obtained by the selective demethylation of 7-chloro-4,6-dimethoxycoumaran-3-one (IX) using a modification of the procedure employed in the demethylation of griseofulvin by Arkley, *et al.*[4]

The structure of the hydroxycoumaranone (X) was established by chemical and spectral evidence. Oxidative degradation of X in anticipation of obtaining the known[7] 3-chloro-2,6-dihydroxy-4-methoxybenzoic acid proved unsuccessful. Our inability to isolate a product from this direct oxidation appears to be consistent with the observation of Molho[8]; according to his interpretation, the corresponding salicylic acid derivative could not be obtained from a 4-hydroxy-substituted coumaranone because it is destroyed under the prevailing reaction conditions. Since our attempts to oxidize 7-chloro-4,6-dimethoxycoumaran-3-one (IX) met with success and yielded the expected 3-chloro-2-hydroxy-4,6-dimethoxybenzoic acid[9] (XIV), we were led to the following approach. Both the hydroxycoumaranone (X) and the known[4] 7-chloro-4-hydroxy-6,2′-dimethoxy-6′-methylgris-2′-ene-3,4′-dione (V), upon conversion to the respective ethyl ether derivatives (XV and XVI[4]) and subsequent oxidation with potassium permanganate, yielded the same acid, 3-chloro-6-ethoxy-2-hydroxy-4-methoxybenzoic acid (XVII), as confirmed by the melting and mixture melting points of the acids as well as their superimposable infrared spectra, and the elemental analyses of the acid and its anilide derivative (XVIII). Moreover, had the hydroxycoumaranone been the isomeric 7-chloro-6-hydroxy-4-methoxycoumaran-3-one, oxidative degradation of its ethyl ether derivative would have afforded the known 3-chloro-4-ethoxy-2-hydroxy-6-methoxybenzoic acid,[10] mp 179–181° dec.



X



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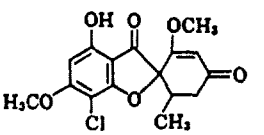
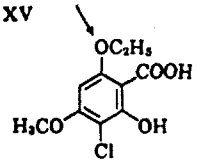
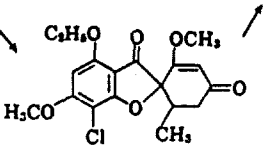


V



XVII



XVI

(1) This investigation was supported by Research Contract No. DA-49-193-MD-2636 from the U. S. Army Medical Research and Development Command, Washington, D. C.

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TABLE I

ULTRAVIOLET ABSORPTION CHARACTERISTICS OF 7-CHLORO-4-HYDROXY-6-METHOXYCOUMARAN-3-ONE (X) AND SELECTED REFERENCE COMPOUNDS

Ref compd[a]	K-band: In 95% EtOH Found[b]		In 95% EtOH with 2% HCl Ref[a]		In 95% EtOH with 2% HCl Found[b]		In aqueous 0.1 *N* NaOH Ref[a]		In aqueous 0.1 *N* NaOH Found[b]	
	log ε	λ_{max}, mμ	log ε	λ_{max}, mμ	log ε	λ_{max}, mμ	log ε	λ_{max}, mμ	log ε	λ_{max}, mμ
XIX, R_1 = OH; R_2 = OCH_3; R_3 = H	4.33	283	4.31	280	4.31	284	4.35	285	4.25	284
XX, R_1 = OCH_3; R_2 = OH; R_3 = H	...	...	4.35	284	...	...	4.53	318	...	...

[a] Summarized from a paper by Duncanson, *et al.*[10] [b] For 7-chloro-4-hydroxy-6-methoxycoumaran-3-one (X).

We obtained additional confirmation for the structure of X by determining its ultraviolet absorption characteristics (see Table I). It can be seen that the spectral characteristics of X are in excellent agreement with those reported by Duncanson[10] for the similarly substituted 4-hydroxy-6-methoxycoumaran-3-one (XIX), and are consistent with the observations of Cram and Cranz[11] on the spectra of *o*- and *p*-hydroxyacetophenones in ionizing and nonionizing media. Compound X must be a 4-hydroxycoumaran-3-one since its K-band, like that of Duncanson's 4-hydroxy derivative, has maxima of similar intensity and wavelength in both ionizing as well as nonionizing media; those of Duncanson's 6-hydroxycoumaran-3-one occur at considerably longer wavelengths and with significantly greater intensity in ionizing media.[10]

Compounds VI, VII, and VIII were prepared by the interaction of X with XI, XII, and XIII in dimethylformamide in the presence of potassium carbonate.

Evaluation of Repellency.—The compounds synthesized have been subjected to screening for mosquito repellency by Dr. C. N. Smith and Mr. H. K. Gouck of the Entomology Research Division, U. S. Department of Agriculture at Gainesville, Fla., by their standardized test.[12] Preliminary tests have shown that the six compounds evaluated possessed no repellent activity against *Aedes aegypti* mosquitoes.

Experimental Section[13,14]

3-(N,N-Diethylcarbamoyl)benzyl Bromide (XII).—To an ice-cold solution of 61.4 g (0.221 mole) of 3-bromomethylbenzoyl bromide[15] (XXI) in 200 ml of anhydrous benzene, a solution of diethylamine (32.3 g, 0.442 mole) in 150 ml of anhydrous benzene was added dropwise. After the reaction mixture was stirred at room temperature for 3 hr, the precipitated diethylamine hydrobromide was removed by filtration and washed with anhydrous benzene. The filtrate and washing were combined and the solvent was removed under reduced pressure. The residual oily liquid (57.0 g, 95.5%) was used without further purification after drying at 100° (0.5 mm) for 3 hr, since it decomposed during all attempts to purify it by vacuum distillation.

8-Bromo-2,6-dimethyloctene-2 (citronellyl bromide) (XIII) was prepared according to the procedure reported for the synthesis of 5-bromo-2-pentene by Goering and co-workers.[16] Phosphorus tribromide (24.5 g, 0.090 mole) was added slowly to a cold solution of citronellol (31.2 g, 0.200 mole) ($[\alpha]^{26}D$ +1.2981° (neat, *l.* = 1 dm), d^{26} 0.8557) and pyridine (6.32 g, 0.090 mole), maintaining the reaction temperature below −20°; the reaction mixture was stirred for 0.5 hr subsequently. The crude product (38.3 g) obtained by a preliminary distillation under reduced pressure was dissolved in ether and washed successively with ice water, 5% $NaHCO_3$, and saturated NaCl solution. After drying (Na_2SO_4), the ether was removed by distillation, and fractionation through a 22.5-cm Vigreux column yielded a colorless liquid (31.8 g, 72.6%), bp 104–108° (5–6 mm), $n^{25}D$ 1.4764, $[\alpha]^{25}D$ −2.9182° (neat, *l.* = 1 dm), d^{25} 1.0946; lit.[6] bp 111° (12 mm), $n^{20}D$ 1.4756, $[\alpha]^{20}D$ −6.93°, d^{20} 1.1105.

7-Chloro-4-hydroxy-6,2′-dimethoxy-6′-methylgris-2′-ene-3,4′-dione (V) was synthesized by the method of Arkley and co-workers.[4] The analytical sample, obtained as the monohydrate by recrystallization from aqueous acetic acid and aqueous methanol, melted at 113.8–115.0° (fusion) in accordance with the observation of Boothroyd, *et al.*[17] While Arkley and co-workers[4] reported the melting point as 140–143°, their value for the specific rotation was in agreement with our findings, $[\alpha]^{25}D$ +322.4° (*c* 1.250, acetone).

Anal. Calcd for $C_{16}H_{19}ClO_6 \cdot H_2O$: C, 53.87; H, 4.80; Cl, 9.94. Found: C, 53.78; H, 4.78; Cl, 10.15.

Prior to use as a reactant in the synthesis of I, II, III, and XVI, this material was dried at 100° (2 mm) for 3 hr.

7-Chloro-4-hydroxy-6-methoxycoumaran-3-one (X).—To a refluxing mixture of 27.8 g (0.110 mole) of iodine in 80 ml of anhydrous ether and 100 ml of anhydrous benzene, 5.6 g (0.230 g-atom) of magnesium turnings was added gradually. The reaction mixture was refluxed for 4 hr and was allowed to stand overnight at room temperature. The clear solution obtained by decantation from this reaction mixture was added slowly to 27.8 g (0.122 mole) of 7-chloro-4,6-dimethoxycoumaran-3-one[18,19] (IX) in 2 l. of refluxing anhydrous benzene. After refluxing for 24 hr, the solvent was removed by distillation under reduced pressure. The residue was treated with 300 ml of 3 *N* HCl and the resulting yellow solid was treated with 150 ml of boiling chloroform. Upon cooling, the resulting solid was filtered off and suspended in 200 ml of boiling dioxane; to this mixture 300 ml of 3 *N* HCl, and subsequently, 200 ml of water were added. The yellow solid so obtained crystallized in the form of yellow needles (12.2 g, 46.6%) from 88% dioxane–water; recrystallized from 85% dioxane–water, it melted at 241.5–242.7° dec; λ_{max}^{EtOH} 210 mμ (ε 20,200), 235 (18,200), 283 (21,600), 325 (4200); $\lambda_{max}^{EtOH-2\%\ HCl}$ 210 mμ (ε 20,000), 236 (17,800), 284 (20,200), 325 (4200); $\lambda_{max}^{0.1N\ NaOH}$ 219 mμ (ε 17,600), 240 (17,000), 284 (17,600), 355 (6200); $\lambda_{max}^{CHCl_3}$ 5.90 μ (C=O).

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(13) The authors acknowledge the technical assistance of Miss Linda F. Lorenzen and Miss Patricia J. Ward.

(14) Melting points were determined with a Swissco melting point apparatus containing silicone fluid and are corrected. Boiling points are uncorrected. Ultraviolet spectra and infrared spectra were obtained, respectively, with the Perkin-Elmer Model 202 and 137B spectrophotometers. A Rudolph Model 62 polarimeter was employed to determine optical rotations. Analyses were performed by Drs. G. Weiler and F. B. Strauss, Oxford, England.

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Anal. Calcd for $C_9H_7ClO_4$: C, 50.37; H, 3.29; Cl, 16.52; OCH_3, 16.02. Found: C, 50.32; H, 3.36; Cl, 16.30; OCH_3, 15.92.

3-Chloro-2-hydroxy-4,6-dimethoxybenzoic Acid (XIV).—To a solution of 2 g (8.75 mmoles) of 7-chloro-4,6-dimethoxycoumaran-3-one (IX) in 700 ml of anhydrous acetone, 8 g of powdered $KMnO_4$ was added. The mixture was stirred at room temperature for 24 hr, and the resulting brown solid obtained by filtration was washed with acetone, dried at 120° for 15 min, and ground with 40 ml of 10% NH_4OH solution. The filtrate and washing were combined and added to 100 g of crushed ice, and this mixture was carefully acidified with concentrated H_2SO_4. The gelatinous-type precipitate was filtered off, dried, and recrystallized from ethyl acetate. The resulting tan needles (0.51 g) melted at 222.0–223.0° dec in accordance with the literature.[3]

7-Chloro-4-ethoxy-6-methoxycoumaran-3-one (XV).—To a mixture of 30 g of anhydrous K_2CO_3 and 4.29 g (0.020 mole) of 7-chloro-4-hydroxy-6-methoxycoumaran-3-one (X) in 120 ml of dimethylformamide, a solution of ethyl bromide (3.27 g, 0.030 mole) in 40 ml of dimethylformamide was added. The reaction mixture was slowly heated to 65° and maintained at this temperature for 5 hr. It was then added to 800 ml of ice water, and the resulting red precipitate was washed with water. Recrystallization from aqueous ethanol gave orange-red needles (3.1 g, 63.9%); after recrystallization from 95% ethanol, a melting point of 180.7–182.2° dec was obtained; λ_{max}^{EtOH} 210 mμ (ε 18,927), 235 (18,200), 286 (19,656), 322 (5096); $\lambda_{max}^{CHCl_3}$ 5.88 μ (C=O).

Anal. Calcd for $C_{11}H_{11}ClO_4$: C, 54.45; H, 4.57; Cl, 14.61. Found: C, 54.58; H, 4.58; Cl, 14.70.

7-Chloro-4-ethoxy-6,2′-dimethoxy-6′-methylgris-2′-ene-3,4′-dione (XVI) was prepared from 10.16 g (0.030 mole) of 7-chloro-4-hydroxy-6,2′-dimethoxy-6′-methylgris-2′-ene-3,4′-dione (V) according to the procedure used in the synthesis of XV. The white solid product (10.1 g, 91.7%) crystallized from benzene–ether in white needles: mp 213.0–213.5°; $[\alpha]^{25}D$ +322.26° (*c* 1.523, acetone); λ_{max}^{EtOH} 218 mμ (ε 23,109), 235 (22,925), 291 (23,659), 328 (5685); $\lambda_{max}^{CHCl_3}$ 5.85 (C=O), 6.02 μ (COC=C); lit.[4] mp 211–213°, $[\alpha]D$ +324°.

3-Chloro-6-ethoxy-2-hydroxy-4-methoxybenzoic Acid (XVII). A. From the Oxidation of 7-Chloro-4-ethoxy-6-methoxycoumaran-3-one (XV).—Compound XV (2.0 g, 8.24 mmoles) was oxidized by the procedure described for oxidation of IX. The product (0.47 g), recrystallized from ethyl acetate, melted at 210.0–211.7° dec; λ_{max}^{KBr} 3.15 (OH), 3.77 (bonded OH), 5.92 μ (C=O).

Anal. Calcd for $C_{10}H_{11}ClO_5$: C, 48.70; H, 4.50; Cl, 14.38. Found: C, 48.62; H, 4.55; Cl, 14.29.

B. From the Oxidation of 7-Chloro-4-ethoxy-6,2′-dimethoxy-6′-methylgris-2′-ene-3,4′-dione (XVI).—A solution of 8.0 g (0.022 mole) of XVI in 1.8 l. of anhydrous acetone was treated with 32 g of powdered $KMnO_4$ by the procedure described for the oxidation of XV. The acid obtained from this reaction melted at 204.7–206.7° dec after recrystallization from ethyl acetate; mixture melting point with the acid obtained in part A above was 207.7–209.0° dec. The infrared spectrum (in KBr) was superimposable on that of the acid obtained in part A.

3-Chloro-6-ethoxy-2-hydroxy-4-methoxybenzanilide (XVIII) was prepared in the customary manner from 0.40 g of 3-chloro-6-ethoxy-2-hydroxy-4-methoxybenzoic acid (XVII) and 1 ml of aniline. Recrystallization from benzene yielded 0.092 g of silky white needles, mp 210.0–210.5°, λ_{max}^{KBr} 3.03 (NH, OH) and 6.08 μ (NC=O).

Anal. Calcd for $C_{16}H_{16}ClNO_4$: C, 59.73; H, 5.01; Cl, 11.02; N, 4.35. Found: C, 59.68; H, 5.30; Cl, 11.08; N, 4.53.

7-Chloro-6,2′-dimethoxy-4-(*p*-methoxybenzyloxy)-6′-methylgris-2′-ene-3,4′-dione (I).—A benzene solution of freshly prepared and vacuum dried (at 25°) crude *p*-methoxybenzyl bromide (XI, prepared from 3.45 g (0.025 mole) of anisyl alcohol) was added slowly to a stirred mixture of 6.77 g (0.020 mole) of anhydrous 7-chloro-4-hydroxy-6,2′-dimethoxy-6′-methylgris-2′-ene-3,4′-dione (V), 25 g of anhydrous K_2CO_3, and 200 ml of anhydrous acetone. After the reaction mixture was refluxed for 16 hr, the solid separated by filtration was washed with two 40-ml portions of boiling acetone and the filtrate and washings were combined and concentrated to dryness. The resulting solid product (8.5 g, 92.5%) was recrystallized from dioxane–absolute ethanol; it melted at 201.6–202.2° dec; $[\alpha]^{26}D$ +246.32° (*c* 1.224, dioxane); λ_{max}^{EtOH} 231 mμ (ε 36,712), 292 (22,027), 335 (6425); $\lambda_{max}^{CHCl_3}$ 5.82 (C=O), 6.0 μ (COC=C).

Anal. Calcd for $C_{24}H_{23}ClO_7$: C, 62.82; H, 5.05; Cl, 7.73. Found: C, 62.69; H, 4.83; Cl, 8.02.

7-Chloro-4-[3-(N,N-diethylcarbamoyl)benzyloxy]-6,2′-dimethoxy-6′-methylgris-2′-ene-3,4′-dione (II) was prepared from V (3.39 g, 0.010 mole) and XII (3.0 g, 0.011 mole) by the procedure described for the synthesis of I. The crude product (4.7 g, 89.0%), recrystallized from benzene, gave white crystalline plates: mp 191.0–192.0°; $[\alpha]^{26}D$ +215.41° (*c* 1.012, acetone); λ_{max}^{EtOH} 214 mμ (ε 35,641), 235 (29,569), 292 (21,912), 330 (5808); $\lambda_{max}^{CHCl_3}$ 5.85 (C=O), 6.02 μ (COC=C).

Anal. Calcd for $C_{28}H_{30}ClNO_7$: C, 63.69; H, 5.73; Cl, 6.72; N, 2.65. Found: C, 63.61; H, 5.81; Cl, 6.61; N, 2.51.

7-Chloro-6,2′-dimethoxy-4-(3,7-dimethyl-6-octenyl-1-oxy)-6′-methylgris-2′-ene-3,4′-dione (III) was prepared from V (6.8 g, 0.020 mole) and XIII (4.6 g, 0.021 mole) by the procedure described for the synthesis of I except that dimethylformamide was employed as the solvent and the reaction temperature was held at 25° for 1 hr and 70° for 4 hr. The crude product (7.8 g, 80.0%) crystallized from 80% methanol in the form of white crystalline plates: mp 136.5–137.5°; $[\alpha]^{26}D$ +252.33° (*c* 0.988, acetone); λ_{max}^{EtOH} 218 mμ (ε 24,327), 235 (23,135), 291 (22,658), 327 (5963); $\lambda_{max}^{CHCl_3}$ 5.82 (C=O), 6.02 μ (COC=C).

Anal. Calcd for $C_{26}H_{33}ClO_6$: C, 65.47; H, 6.97; Cl, 7.43. Found: C, 65.42; H, 6.91; Cl, 7.60.

7-Chloro-6-methoxy-4-(*p*-methoxybenzyloxy)coumaran-3-one (VI) was prepared from X (2.15 g, 0.010 mole) and XI (2.61 g, 0.013 mole) by the procedure described for the synthesis of III. The solid product (3.1 g, 92.6%) was recrystallized from dioxane giving the analytical sample: mp 182.6–183.2° dec; λ_{max}^{EtOH} 232 mμ (ε 26,446), 285 (17,408), 322 (5021); $\lambda_{max}^{p\text{-dioxane}}$ 236 mμ (ε 23,768), 283 (18,914), 317 (5858); λ_{max}^{KBr} 5.87 μ (C=O).

Anal. Calcd for $C_{17}H_{15}ClO_5$: C, 60.99; H, 4.52; Cl, 10.59. Found: C, 60.94; H, 4.76; Cl, 10.55.

7-Chloro-4-[3-(N,N-diethylcarbamoyl)benzyloxy]-6-methoxycoumaran-3-one (VII) was prepared from X (2.15 g, 0.010 mole) and XII (2.97 g, 0.011 mole) by the procedure described for the synthesis of III. The product (2.9 g, 71.8%) was recrystallized from ethyl acetate giving cream-colored microcrystalline needles: mp 141.0–143.0° dec; λ_{max}^{EtOH} 208 mμ (ε 37,157), 235 (26,252), 286 (19,790), 320 (6866); $\lambda_{max}^{CHCl_3}$ 5.87 (C=O), 6.18 μ (NC=O).

Anal. Calcd for $C_{21}H_{22}ClNO_5$: C, 62.45; H, 5.49; Cl, 8.78; N, 3.47. Found: C, 62.69; H, 5.46; Cl, 9.00; N, 3.50.

7-Chloro-6-methoxy-4-(3,7-dimethyl-6-octenyl-1-oxy)coumaran-3-one (VIII) was prepared from X (2.15 g, 0.010 mole) and XIII (2.63 g, 0.012 mole) by the procedure described for the synthesis of III. The product (2.1 g, 59.5%) was recrystallized from methanol, yielding the analytical sample: mp 75.2–76.0°; λ_{max}^{EtOH} 209 mμ (ε 20,643), 235 (18,349), 285 (18,173), 320 (4940); $\lambda_{max}^{CHCl_3}$ 5.85 μ (C=O).

Anal. Calcd for $C_{19}H_{25}ClO_4$: C, 64.67; H, 7.14; Cl, 10.05. Found: C, 64.67; H, 7.05; Cl, 10.05.

Acknowledgments.—We gratefully acknowledge the generous supply of griseofulvin furnished by Ayerst Laboratories, McNeil Laboratories, Inc., and by the Schering Corp. We also wish to express our appreciation to Dr. C. N. Smith and Mr. H. K. Gouck of the Entomology Research Division, U. S. Department of Agriculture, for evaluating the mosquito repellency of the compounds reported in this communication.

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13. ABSTRACT

The subject investigation constitutes a continuation of our previous studies directed toward the development of reliable prophylactic agents against pathogenic or physical impairments inflicted through the skin. While our experimental design encompasses studies which are anticipated to yield information for the development of moieties possessing keratinization-enhancing, sunlight-induced-erythemogenesis-preventing, antidermatophytic and antibacterial properties, our primary targets are dependable and safe agents with long-lasting insect-repellent efficacy. (U).

In order to accomplish the objectives set forth above, we have undertaken studies on the structural requirements and physicochemical characteristics associated with a compound's capacity (a) for anchoring to dermal tissue constituents following its topical administration and/or (b) for enhancing its localization in dermal tissue following its parenteral, and even peroral, administration. It is our endeavor to integrate either of these, with molecular features known to possess insect-repellency, into new hybrid molecules. These new synthetic moieties would be expected to be long-lasting insect-repellent agents whether they be applied topically or administered systemically. (U).

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14 KEY WORDS: Key words are technically meaningful terms or short phrases that characterize a report and may be used as index entries for cataloging the report. Key words must be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical context. The assignment of links, rules, and weights is optional.

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In conformance with these considerations, within the period covered by This Report: (a) The information developed in our laboratories during the preceding year was supplemented with exhaustive literature surveys, and the premises of our rationale delineated in our Report No. 1. were reinforced and expanded. (b) Six additional new target compounds were synthesized, characterized and their postulated structures confirmed. The novel hybrid molecules, reported for the first time, are: tetrahydropyran-2-yl ester of 4-(10-carboxydecyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (compound A028), 4-(10-carboxydecyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (compound A029), tetrahydropyran-2-yl ester of 4-(9-carboxynonyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (compound A032), 4-(9-carboxynonyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (compound A033), tetrahydropyran-2-yl ester of 4-(7-carboxyheptyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (compound A035), 4-(7-carboxyheptyl-1-oxy)-7-chloro-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione (compound A036). Additional quantities of the first six new compounds, reported in the preceding communication, were prepared for further insect-repellent and antidermatophytic evaluations. (c) Instrumentation has been further refined and the previously described techniques have been advanced permitting use of a uniform monomolecular-film system for the evaluation of all compounds studied in our laboratories. The interactions of our synthetic entities with stearic acid, cholesterol, lecithin and other skin constituents have been, or are expected to be, studied. We consider ourselves fortunate that our very first attempts in designing the proposed new hybrid molecules yielded compounds some of which registered measurable interaction with substances known to be components of, and to have prominent functions in, dermal tissue. (d) The insect-repellent properties of our compounds were studied by Doctor Carroll N. Smith (Investigations Leader, Entomology Research Division, U.S.D.A.) and Mr. Philip Kashin (Associate Biochemist, Life Sciences Research, IIT Research Institute). Here, too, our initial efforts resulted in novel compounds, some of which, among those already evaluated, registered measurable insect-repellent characteristics. While we are most interested in developing agents surpassing in effectiveness those currently in use, immediately, we are concerned with the relative potency of our synthetic entities with respect to each other; the importance of this approach in finding significant leads to the solution of the more practical aspects of the problem cannot be overestimated. We are therefore not particularly concerned, at this time, with the fact that none among our first six compounds yielded favorable results in U.S.D.A.'s standard mosquito-repellent screening test for practical application. (e) The efficacy of our compounds in inhibiting dermatophytic organisms was evaluated by Doctor Robert G. Crounse (Chairman, Division of Dermatology, Johns Hopkins University School of Medicine). It is salient that, in this instance also, rather significant responses were observed. Among our compounds, those already evaluated required

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a higher concentration than the standard antidermatophytic agent. In addition to considering that we are dealing with the products of the initial phase of our exploratory efforts, and are primarily seeking leads, we feel that, if our compounds should possess enhanced localization characteristics in dermal tissue, the latter could render them therapeutically more effective. Doctor Crounse is also in the process of determining the range of the spectrum of antidermatophytic activity for each of our compounds. (U).